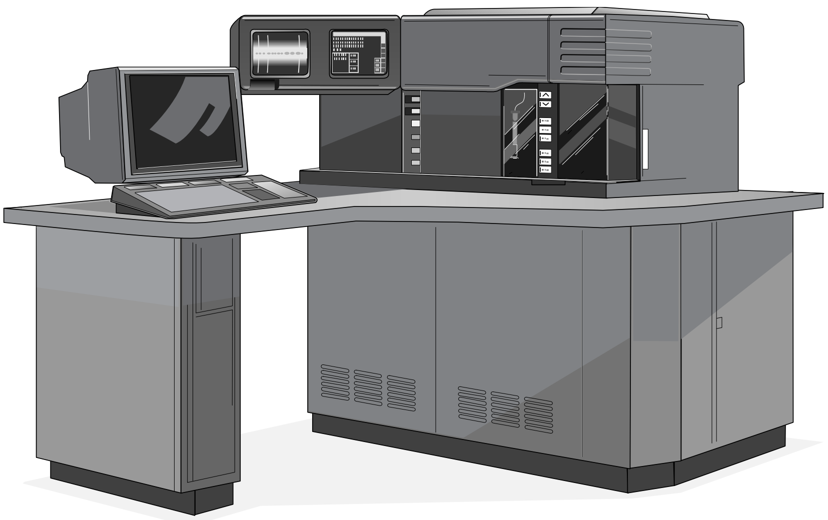
COULTER® EPICS® Elite Flow Cytometer

Service Manual



PN 4237003C (December 2001)

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LEGAL NOTICES

Beckman Coulter, Inc. makes no representation that, upon furnishing this service manual, the holder of the manual will have the necessary technical capabilities and know-how to properly troubleshoot and repair any of the equipment specified in the manual. Beckman Coulter, Inc. assumes no liability whatsoever, including consequential and incidental damages, resulting from improper operation of Beckman Coulter instruments after maintenance of Beckman Coulter instruments has been performed by persons not employed by Beckman Coulter, Inc. Furthermore, Beckman Coulter, Inc. assumes no liability whatsoever for any personal injury or property damage resulting from maintenance and/or repair of Beckman Coulter instruments performed by persons not employed by Beckman Coulter, Inc.

READ ALL PRODUCT MANUALS AND CONSULT WITH BECKMAN COULTER-TRAINED PERSONNEL BEFORE ATTEMPTING TO OPERATE INSTRUMENT.

HAZARDS AND OPERATIONAL PRECAUTIONS AND LIMITATIONS

WARNINGS, CAUTIONS, and IMPORTANTS alert you as follows:

WARNING - Can cause injury.
CAUTION - Can cause damage to the instrument.
IMPORTANT - Can cause misleading results.

Beckman Coulter, Inc. urges its customers to comply with all national health and safety standards such as the use of barrier protection. This may include, but it is not limited to, protective eyewear, gloves, and suitable laboratory attire when operating or maintaining this or any other automated laboratory analyzer.

Initial Issue, System Installation Procedure, 11/91
Software Version 2.0

Revision B, Complete Service Manual, 04/97
Released by CN 303160-0994
Software Version 4.01

Revision C, 12/01
Released by CN 303160-1702
Software Version 4.5
This manual was reformatted to enhance the electronic version. Reformatting the manual changed the appearance of each page, but it did not change the content. Consequently, a revision C manual and a revision B manual with revision C change pages look different, but they contain the same material, page for page. The material in the revision C change pages was updated for hardware and software changes made since the last revision including the Elite 256 Sort option.

Revision C is being released as change pages (PN 4277432). This revision updates and incorporates information released by Service Memos 1382 and 1795, and Service Bulletin 1328.

Changes were made on the following pages 1.2-2, 1.2-9, 2.3-13, 2.5-1, 2.5-2, 2.8-4, 2.9-1, 3.1-1, 3.1-3, 3.9-1, 3.13-1 through 3.13-5, 3.22-1 through 3.22-2, 4.10-1 through 4.10-2, 6.1-1 through 6.1-4, 8.1-1, 8.1-2, 8.1-3, 8.1-6, 8.1-7, 8.1-16, 8.1-18, 8.1-20, 8.1-22, 8.2-5, 8.2-8, 8.2-9, 8.2-11, 8.2-12, 8.2-38, A.2-2, ABBREVIATIONS, TRADEMARKS.

Additionally,

- Installation procedure 3.22 CYTOMETER CPU CARD UPGRADE was replaced with ELITE 256 SORT UPGRADE.
- Heading 4.13, EXTERNAL MEMORY CARD REPLACEMENT was added.
- Heading 7.6, RESETTING CYTOMETER SOFTWARE LOCKUPS was added.
- Under Heading 8.1, MASTER PARTS LIST IN NUMERICAL ORDER BY CATEGORY, Table 6 Cytometer and Table 7 Cytometer were combined. As a result, table numbers 8 and above are decreased by one. For example, Table 8.1-8 is now Table 8.1-7 and so forth.

Since check digits are not used in the VANTIVE or CARES systems, all check digits have been removed from the part numbers in Chapter 8. To avoid confusion when ordering parts, that entire chapter has been added to the change page packet.

Changes that are part of the most recent revision are indicated in the printed copy by a bar in the margin of the amended page.

This document applies to the latest software listed and higher versions. When a subsequent software version affects the information in this document, the changes will be included on minor revision change pages or summarized on a Notice of Information Update form and will be released by service memo.

REVISION STATUS

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ILLUSTRATIONS

1.1 MANUAL DESCRIPTION

Scope

This manual provides the reference information and procedures needed to service and maintain the COULTER EPICS Elite flow cytometer and the COULTER EPICS Elite ESP flow cytometer. It is available both online and in hard copy. The online manual is released on the Service Resource Kit CD-ROM, PN 6417471-1.

This manual does not contain information and/or procedures already covered in other manuals for these instruments.

Use this manual in conjunction with the following Elite customer manuals:

- Reference manual, PN 4235904
- Operator's Guide, 4235932
- Options manual, PN 4235965
- Software manual, PN 4237024
- Special Procedures and Troubleshooting manual, PN 4235931

To service the Computer Workstation, which consists of the central processing unit (CPU), color monitor, keyboard, and mouse, refer to the manual supplied by the manufacturer of the Workstation.

To service the Graphic Printer, refer to the manual supplied by the manufacturer of the printer.

To service a laser, refer to the manual supplied by the manufacturer of the laser.

Any service memo that affects the information in this manual will include a Notice of Information Update form for this manual. The Notice of Information Update form will summarize the changes and will list the specific headings, figures, and tables affected.

Intended Audience

To use this manual effectively, you need the following:

- An operator's knowledge of the Elite
- Service training on the Elite
- A basic understanding of -
  - Cytometry terms and concepts
  - Computer hardware.
- The ability to -
  - Read pneumatic/hydraulic schematics and understand related terminology
  - Read electronic schematics and understand related terminology
  - Use a digital volt meter (DVM) and an oscilloscope
  - Use basic mechanical tools and understand related terminology.

Organization

The material in this manual is organized into eight chapters and two appendices. To make it easier to access the information:

- In the online manual, each page has a Contents button linked to a master table of contents and an Index button linked to an alphabetical index.
- In the printed manual there is a master table of contents at the beginning of the manual, a chapter-specific table of contents at the beginning of each chapter, and an alphabetical index at the end of the manual.
- **CHAPTER 1, INTRODUCTION**, briefly describes this manual and provides essential safety information.
- **CHAPTER 2, INSTRUMENT DESCRIPTION**, introduces the Elite and Elite ESP and describes how each functions.
- **CHAPTER 3, INSTALLATION PROCEDURES**, includes procedures for installing the instrument and any options or upgrades.
- **CHAPTER 4, SERVICE AND REPAIR PROCEDURES**, includes procedures for servicing and repairing the instrument. Each procedure includes a purpose and a list of tools and supplies needed to do the procedure.
- **CHAPTER 5, MAINTENANCE PROCEDURES**, includes procedures for maintaining the instrument.
- **CHAPTER 6, SCHEMATICS**, includes engineering schematics.
- **CHAPTER 7, TROUBLESHOOTING**, includes troubleshooting tables.
- **CHAPTER 8, ILLUSTRATED PARTS LIST**, includes the illustrated parts list.
- **APPENDIX A, QUICK REFERENCE INFORMATION**, includes instrument tolerances and specifications, jumper and switch settings, test points, and connectors.
- **APPENDIX B, FILE PATHS**, provides a block diagram of the software file location.

Format

Most of the material in this manual pertains to both the EPICS Elite flow cytometer and the EPICS Elite ESP flow cytometer. In those cases where the information applies only to the EPICS Elite ESP flow cytometer, that information is referred to as Elite ESP.

Numbering Format

Each chapter of this manual is divided into topics, that are numbered sequentially, beginning at one. The numbering format for the topic heading, which is called the primary heading, is chapter number, decimal point, topic number. For example, the primary heading number for the fifth topic covered in Chapter 4 is 4.5.

The page, figure, and table numbers are linked directly to the primary heading number. For example, Heading 4.5 begins on page 4.5-1; the first figure under Heading 4.5 is Figure 4.5-1; and the first table under Heading 4.5 is Table 4.5-1.

Note: Primary headings always begin on the top of a right page.

Special Headings

Throughout this manual, WARNING, CAUTION, IMPORTANT, ATTENTION, and Note headings indicate potentially hazardous situations and important or helpful information.

Warning

A **WARNING** indicates a situation or procedure that, if ignored, can cause serious personal injury. The word **WARNING** is in bold-faced text in the printed manual and is red in the online manual.

Caution

A **CAUTION** indicates a situation or procedure that, if ignored, can cause damage to equipment. The word **CAUTION** is in bold-faced text in the printed manual and is red in the online manual.

Important

An **IMPORTANT** indicates a situation or procedure that, if ignored, can cause erroneous test results. The word **IMPORTANT** is in bold-faced text in the printed manual and is red in the online manual.

Attention

An **ATTENTION** contains information that is critical for the successful completion of a procedure and/or operation of the instrument. The word **ATTENTION** is in bold-faced text in the printed manual and is red in the online manual.

Note

A Note contains information that is important to remember or helpful in performing a procedure.

Conventions

This manual uses the following conventions to make the material clearer and more concise. An example is given for each explanation:

- *Italics* indicate screen messages, such as *Waste Bottle Full*.
- **Bold** indicates a menu item, such as **Acquisition**.
- `Courier` font indicates text that you must type, such as `INSTALL`.
- The software path to a specific function or screen appears with the double solid-right triangle (▸▸) symbol between succeeding screen options, such as **Acquisition ▸▸ Parameter**.
- Whenever keys are to be pressed in succession, the keys are printed with a space but no punctuation between them, such as [F9] [Enter].
- Whenever keys are to be pressed simultaneously, the keys are printed with a plus sign between them, such as [Ctrl]+[Alt]+[Delete].
- Links to additional information are in blue and are underlined in the online manual. To access the linked information, select the blue, underlined text such as Conventions.

1.2 SAFETY PRECAUTIONS

This section covers safety precautions that you must take whenever you work on the Elite. Additionally, when performing a procedure, always follow any safety precautions included in that procedure, as they supplement the precautions listed in this section.

Electronic

WARNING Risk of electric shock. Be very careful when operating the instrument with safety interlock bypass jumpers installed, as you may be exposed to electric shock. Always REMOVE ALL SAFETY INTERLOCK BYPASS JUMPERS after servicing the instrument.

To protect the operator from personal injury, this instrument is equipped with safety interlock switches that turn off the power when the front or rear cover is removed. Be very careful if you bypass these safety interlocks and operate the instrument with the covers off.

WARNING Risk of personal injury. Rings or other jewelry can contact exposed electronic components, causing personal injury from electronic shock. Remove rings and other metal jewelry before performing maintenance or service on the electronic components of the instrument.

CAUTION Risk of damage to electronic components.

- If you remove or replace a printed circuit card or electronic component while the power is ON, the component may be damaged. To prevent damage to delicate electronic components, turn OFF the power before removing or replacing printed circuit cards and/or components.
- Electrostatic discharge (ESD) can damage disk drives, add-in circuit cards, and other electronic components. If there is a possibility of ESD damage with a procedure, then perform that procedure at an ESD workstation, or wear an antistatic wrist strap attached to a metal part of the chassis connected to an earth ground.

Biological

WARNING Risk of personal injury or contamination. If you do not properly shield yourself before servicing the instrument with the door open, you may be injured or contaminated. To prevent possible injury or biological contamination, you must wear gloves, a lab coat, and eye protection when servicing the instrument with the doors open and/or when working with pathogenic materials.

Use extreme care when working with pathogenic materials. Means must be available to decontaminate the instrument, to ventilate air, and to dispose of waste liquid. Refer to the following publications for further guidance on decontamination:

- Biohazards Safety Guide, 1974, National Institute of Health.
- Classifications of Etiological Agents on the Basis of Hazards, 3d ed., June 1974, Center for Disease Control, U.S. Public Health Service.

Laser

Laser Beam Hazards

The laser is a unique light source that shows characteristics different from those of conventional light sources. The safe use of the lasers depends upon familiarity with the instrument and with the properties of coherent, intense beams of light.

Because the Elite contains at least one laser, the customer should keep the instrument isolated from nonlaser instruments. The customer should also keep a copy of ANSI Standard 136.1, SAFE USE OF LASERS, near the instrument for ready reference. Copies are available from:

American National Standards Institute
1430 Broadway
New York, NY 10018

WARNING Risk of personal injury. The laser beam can cause eye damage if viewed either directly or indirectly from reflective surfaces (such as a mirror or shiny metal surface). To prevent eye damage, avoid direct exposure to the beam. Do not view it directly or with optical instruments except with special service tools as directed in this manual.

Eye and skin damage, as well as instrument damage, can be caused by the laser beams. The laser has enough power to ignite substances placed in the beam path, even at a distance. Indirect contact with the laser beam from reflective surfaces (such as jewelry), called specular reflection, might also cause damage.

Follow these precautions:

- Never look directly into the laser light source or at scattered laser light from any reflective surface. Never look down into the beam's source.
- As a precaution against accidental exposure to the output beam or its reflection, personnel performing service or maintenance procedures on the system should wear proper laser safety glasses.
- Do not use lasers in the presence of flammables or explosives; these include volatile substances such as alcohol, solvents, and ether.
- Avoid direct exposure and indirect reflection of the laser beam to your skin.
- Assure that any spectators are not potentially exposed to a hazardous condition.
- Do not leave the laser unattended if there is a chance than an unauthorized person may attempt to use it.

Radiation Hazards

WARNING Risk of radiation exposure. To reduce the risk of exposure to radiation, do not use controls or adjustments or perform any procedures other than those specified in this manual.

In the design and manufacture of the Elite flow cytometer, Beckman Coulter has complied with the requirements governing the use and application of a laser as stipulated in regulatory documents issued by the U.S. Department of Health and Human Services, and by the National Center for Devices and Radiological Health (CDRH). In compliance with these regulatory

documents, every measure has been taken to ensure the health and safety of users, laboratory personnel and service personnel from the possible dangers of laser use. The laser is classified as Class I when it is in the system with the protective housing in place.

CDRH-approved labels are placed near or on those covers that when removed might expose laser radiation. See Figures 1.2-1 through 1.2-10 for the labels and their locations on the lasers:

- Figure 1.2-1 Laser Warning Labels: Coherent Innova 300 Water-Cooled Argon Laser
- Figure 1.2-2 Laser Warning Labels: Innova 90 Water-Cooled Argon Laser
- Figure 1.2-3 Laser Warning Labels: Helium-Cadmium Laser
- Figure 1.2-4 Laser Warning Labels: Omnichrome Helium-Cadmium 74 Laser
- Figure 1.2-5 Laser Warning Labels: Coherent Innova Enterprise Laser
- Figure 1.2-6 Laser Warning Labels: Coherent Innova 305 Laser
- Figure 1.2-7 Laser Warning Labels: Coherent Spectrum Laser
- Figure 1.2-8 Laser Warning Labels: Uniphase Red HeNe Laser
- Figure 1.2-9 Laser Warning Labels: Melles Griot Green HeNe Laser
- Figure 1.2-10 Laser Warning Labels: Sensing Area

Figure 1.2-1 Laser Warning Labels: Coherent® Innova® 300 Water-Cooled Argon Laser

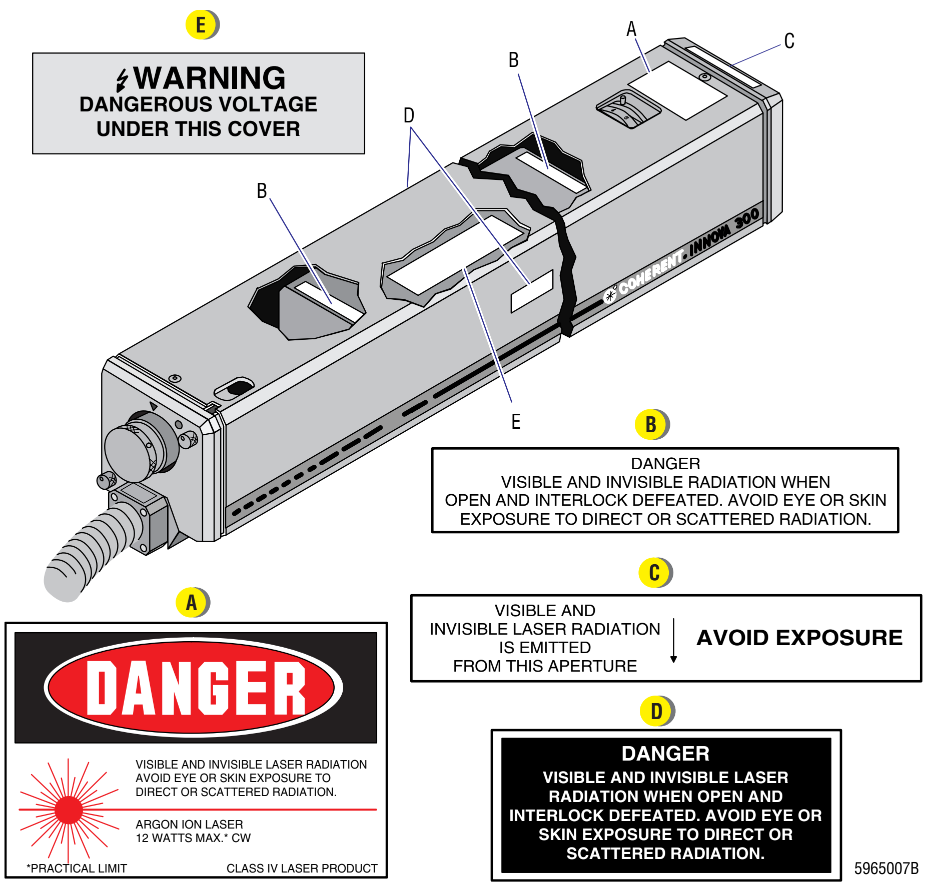


Figure 1.2-2 Laser Warning Labels: Innova 90 Water-Cooled Argon Laser

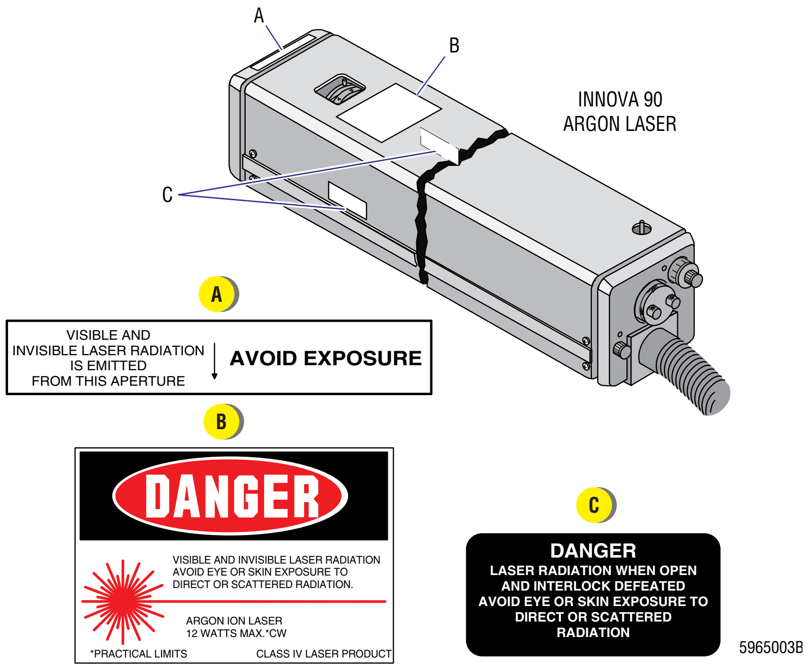


Figure 1.2-3 Laser Warning Labels: Helium-Cadmium Laser

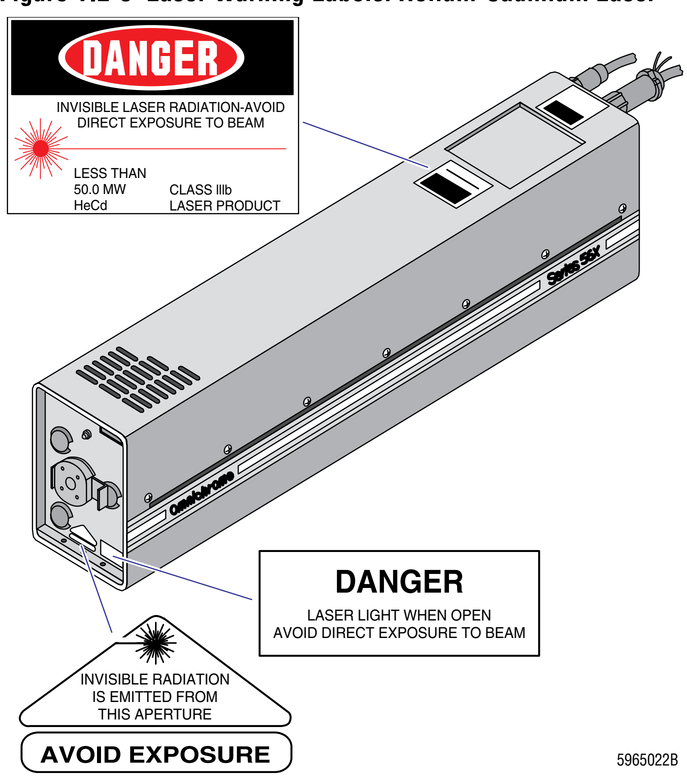


Figure 1.2-4 Laser Warning Labels: Omnichrome Helium-Cadmium 74 Laser

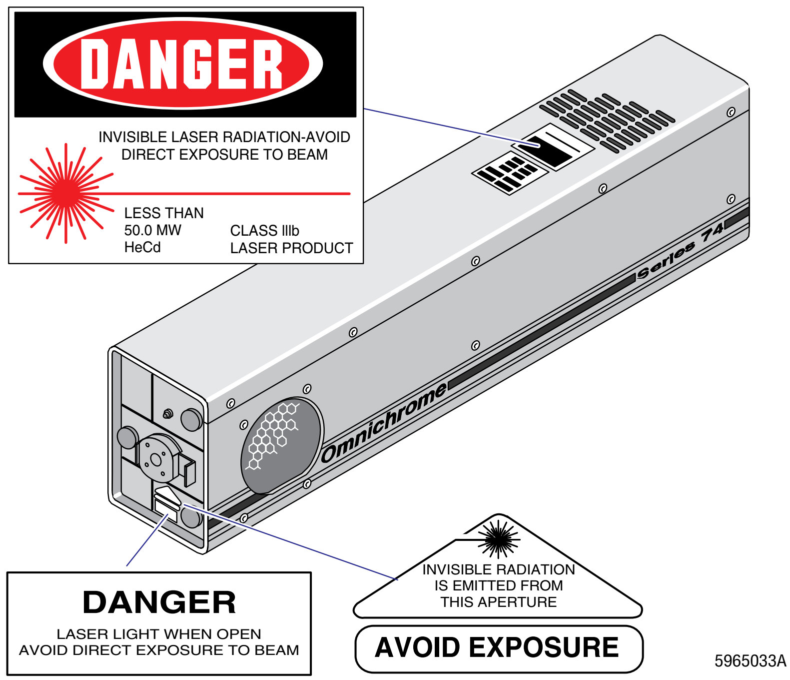


Figure 1.2-5 Laser Warning Labels: Coherent Innova Enterprise Laser

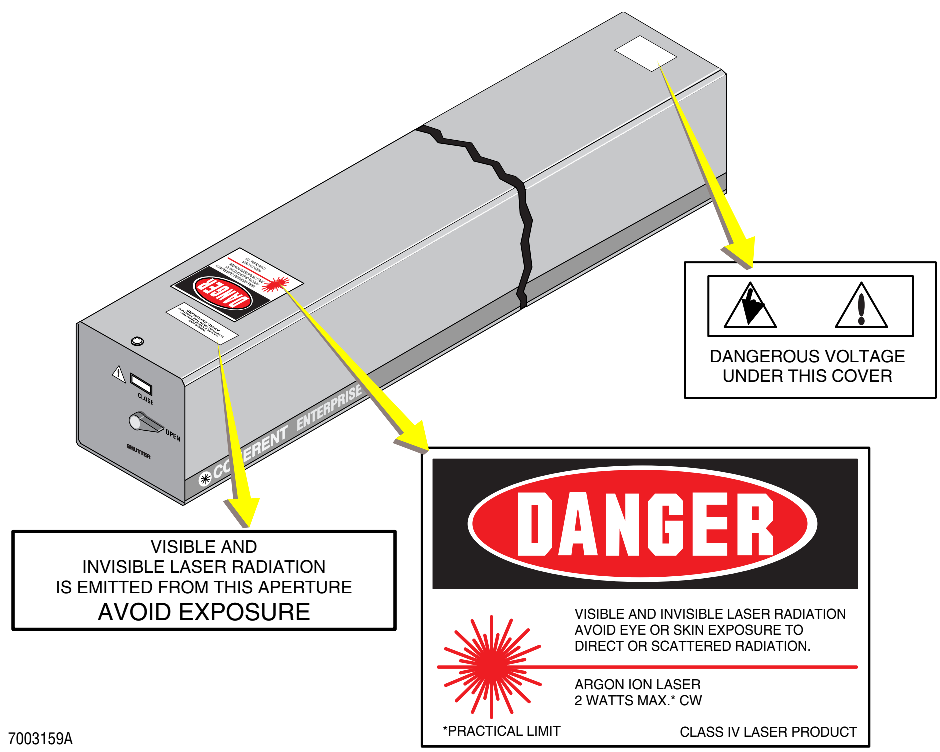


Figure 1.2-6 Laser Warning Labels: Coherent Innova 305 Laser

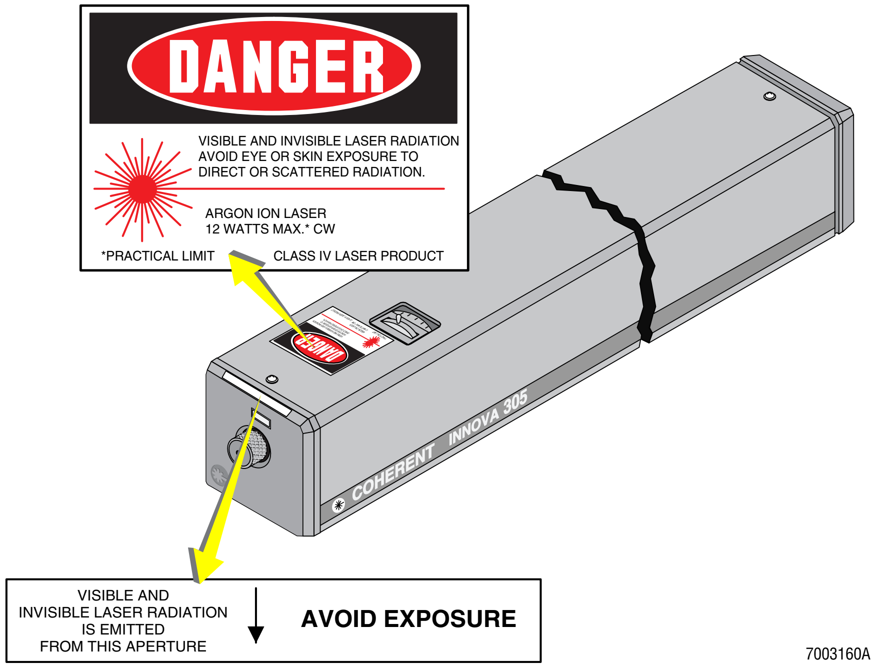


Figure 1.2-7 Laser Warning Labels: Coherent Spectrum Laser

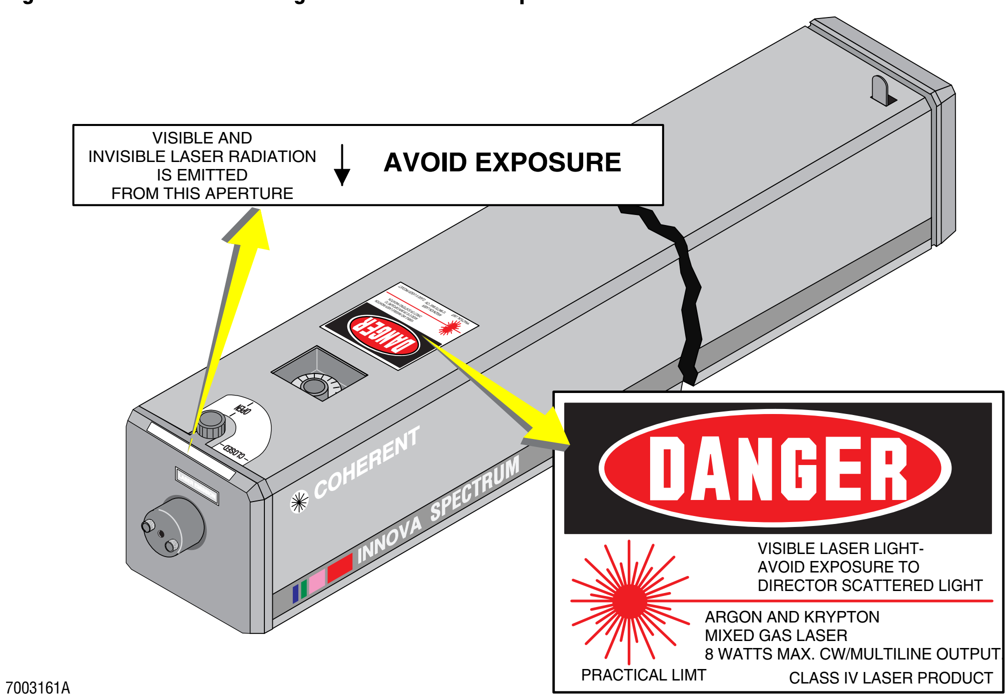


Figure 1.2-8 Laser Warning Labels: Uniphase Red HeNe Laser

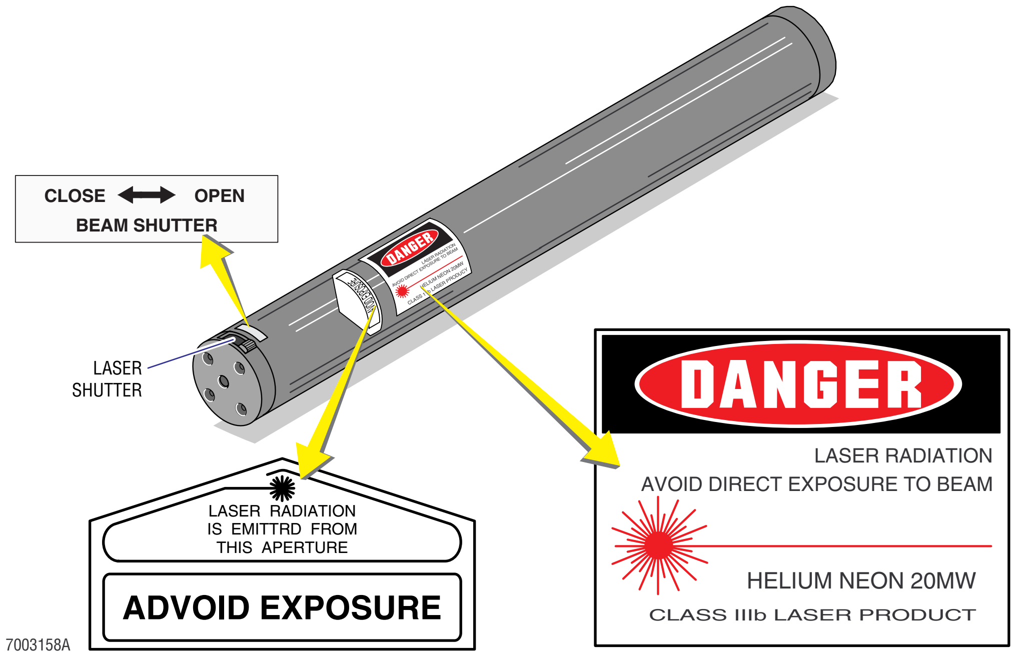


Figure 1.2-9 Laser Warning Labels: Melles Griot Green HeNe Laser

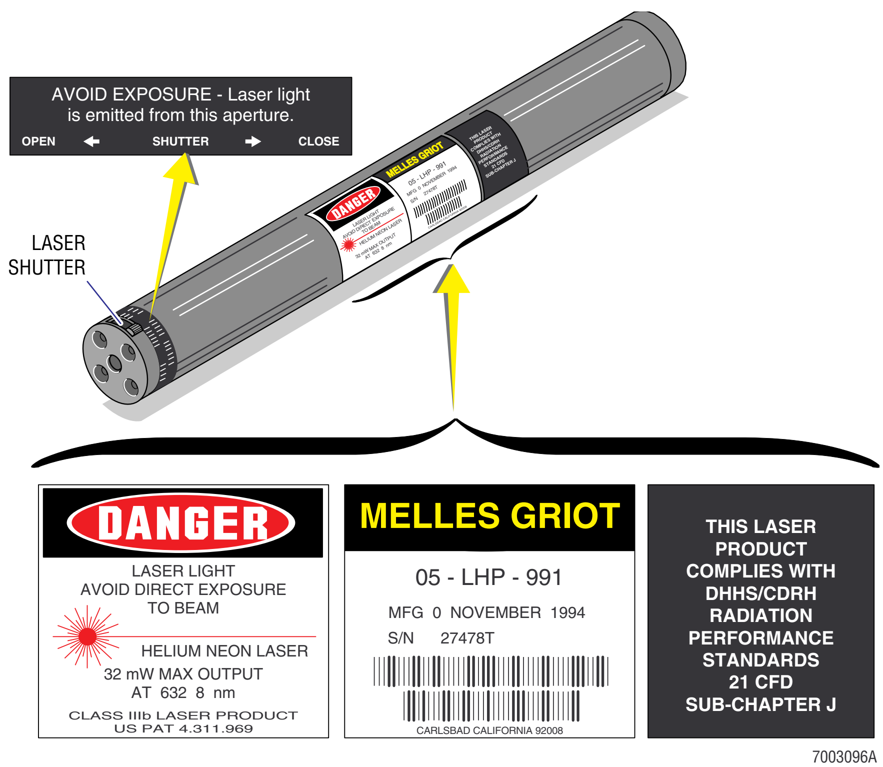
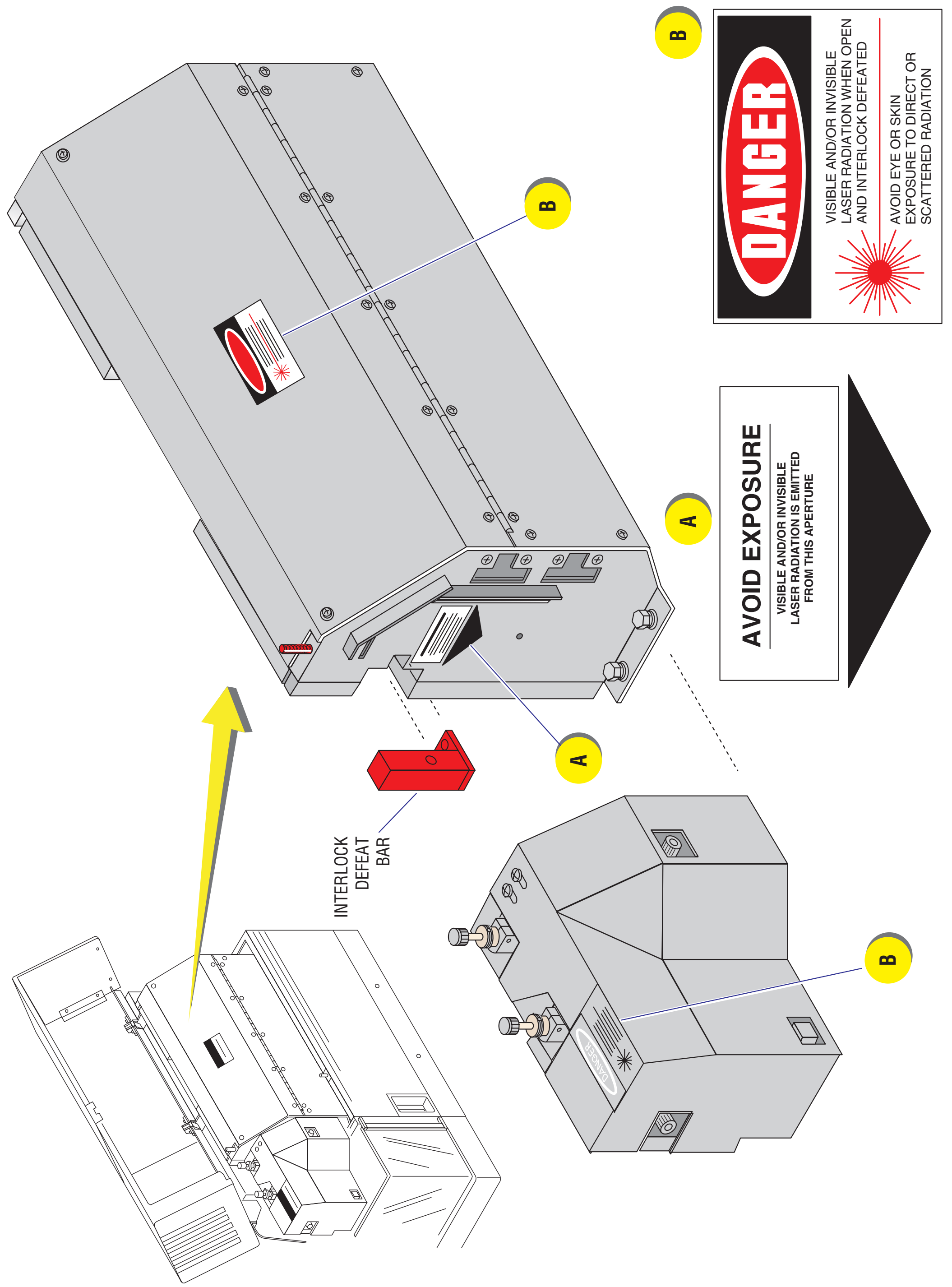


Figure 1.2-10 Laser Warning Labels: Sensing Area



Notify the Customer About Barrier Protection

Bring the following warning to the customer's attention before advising the customer to perform any service, maintenance, troubleshooting, or service procedure on the instrument.

WARNING Risk of personal injury or contamination. If you do not properly shield yourself while performing service, maintenance, and troubleshooting procedures, residual fluids in the instrument could injure or contaminate you. Beckman Coulter recommends that you wear barrier protection, such as appropriate safety glasses, lab coat, and gloves throughout the performance of service, maintenance, and troubleshooting procedures to avoid contact with cleaners and/or residual fluids in the instrument.

ILLUSTRATIONS

TABLES

2.1 INSTRUMENT OVERVIEW

Function

The Elite is a cytometry analyzer that processes flow cytometric measurement and sorting of cells within a sample suspension.

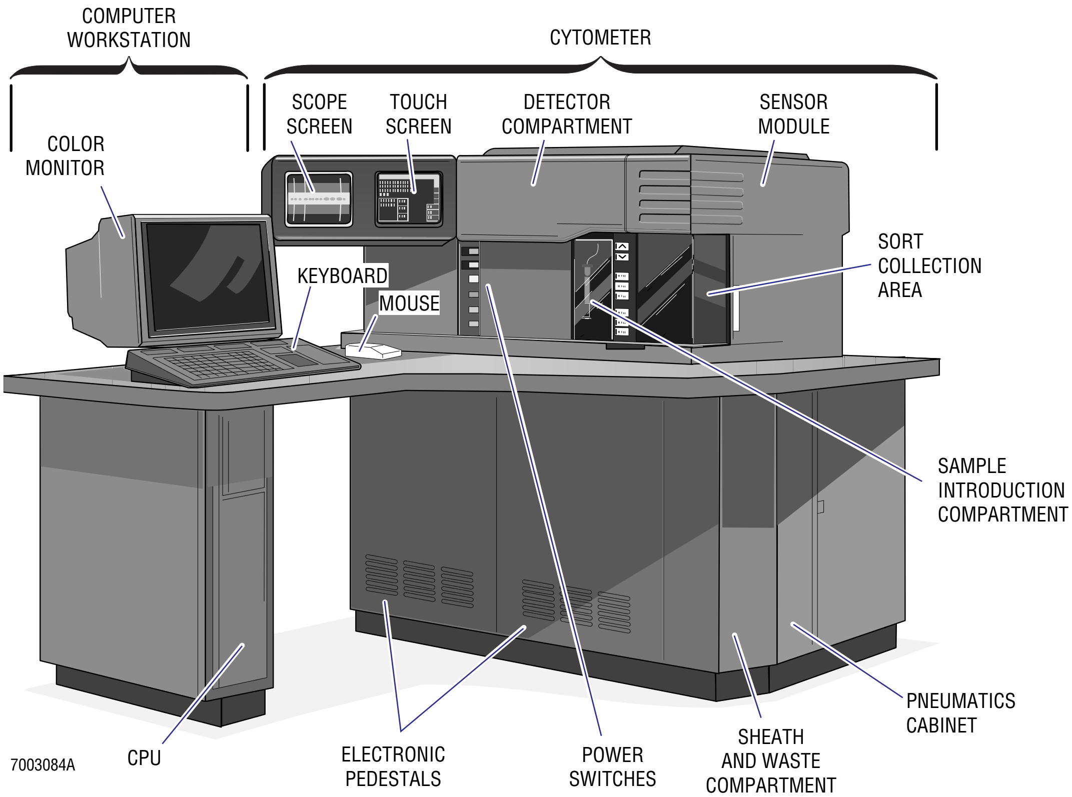
For more information on the Elite's function, refer to Chapters 1 and 3 of the Reference manual.

Description

Main Components

Two main components comprise the Elite: the Cytometer and the Workstation. See Figure 2.1-1

Figure 2.1-1 Elite Components



Subsystems

The Elite is comprised of several subsystems, each of which is described in detail later in this manual. The subsystems include:

- Control and Display subsystems (see Heading 2.5)
  - Multibus subsystem (see Multibus Subsystem under Heading 2.5)
  - Cytometer Computer subsystem (see Cytometer Computer Subsystem under Heading 2.5)

    - Laser Control subsystem (see Laser Control Subsystem under Heading 2.5)
    - Video subsystem (see Video Subsystem, Cytometer under Heading 2.5)
    - Control Pathways subsystem (see Cytometer Control Subsystem under Heading 2.5).
- Acquisition subsystem (see Heading 2.6, ACQUISITION SUBSYSTEM)
- Sort subsystem (see Heading 2.8, SORT SUBSYSTEM)
- Pneumatics subsystem (see Heading 2.9, PNEUMATICS SUBSYSTEM)
- Electronics subsystem (see Heading 2.4, ELECTRONICS SUBSYSTEM).

Physical Specifications

Refer to Chapter 2 of the Reference manual.

Power Requirements

Refer to Chapter 2 of the Reference manual.

Reagents and QC Materials

Refer to Chapter 1 of the Reference manual.

Software

The Elite operates with two software programs, the Elite software and the Cytometer software. For more information on the software, refer to the Software manual.

Fluorescence and Light Scatter Measurement Overview

The system measures the fluorescence and laser light scattered from cells or from other microscopic particles passing through a laser beam. Samples must be single cell or particle suspensions; the system measures up to 10,000 cells per second.

The intensity of fluorescence gives the affinity of the sample for certain dyes, or of the inherent fluorescence of the sample particles.

There are two types of scattered light: forward and side. Forward light scatter correlates with particle size. Side light scatter correlates with the granularity or complexity of a cell's composition.

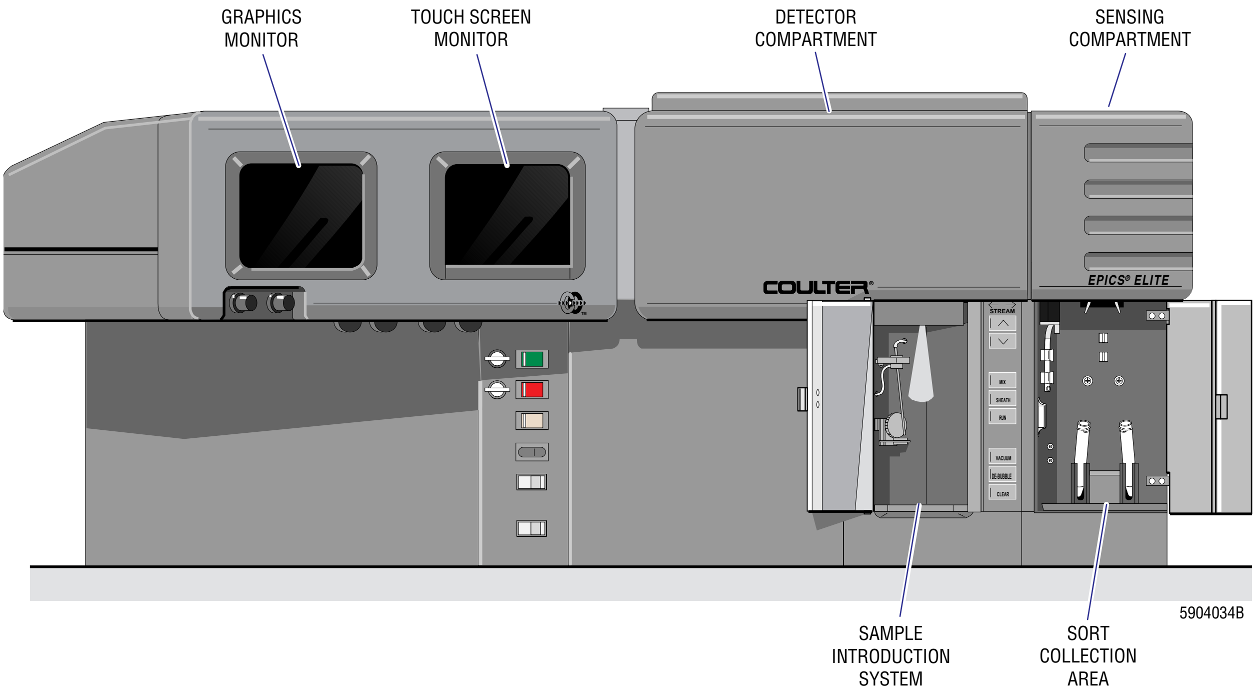
The Elite correlates and stores several measurements of cell characteristics, and graphically displays the distribution of those characteristics within the sample. The system can then sort the cells based on the measured characteristics.

Sensor Area

The Sensor area (Figure 2.1-2) consists of the:

- Sample introduction system
- Sensing compartment
- Sort collection area
- Detector compartment
- Graphics and touch screen monitors.

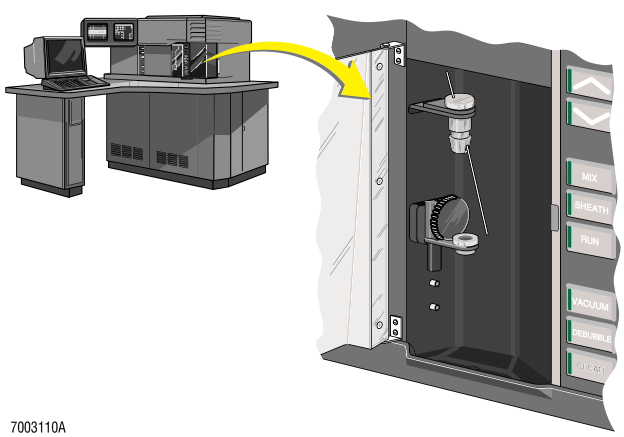
Figure 2.1-2 Sensor Area



Sample Introduction System

The sample stage (Figure 2.1-3) accepts samples for analysis in 12 x 75 mm test tubes. Sample pickup is by positive pressure. Sample flow is guided into the sheath stream at the flow chamber. Sheath fluid flows from a sheath tank in the sheath and waste compartment. An internal compressor pressurizes the sheath. There is a bank of controls to the right of the sample stage that provides manual control of sheath and sample flow.

Figure 2.1-3 Sample Stage



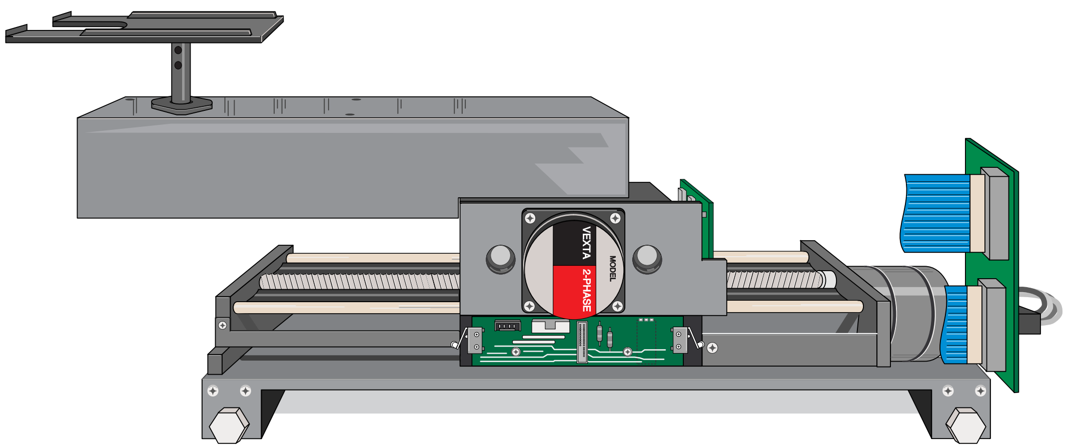
2.2 AUTOCLONE® SORTING OPTION

General

The Autoclone Sorting Option (Figure 2.2-1) is a programmable single-cell deposition system that:

- Sorts a user-defined number of cells per well into a multi-well microculture plate.
- Automates hybridoma screening for research and development of monoclonal antibodies, sterile sorting of bacterial cultures, and screening for high-density monoclonal markers.
- Accepts standard 6, 12, 24, 48, 60, or 96 well microculture plates for automated cell deposition.
- Automates the process of determining the optimum Drops Delay setting through its Sort Matrix function.

Figure 2.2-1 Autoclone Sorting Option Mechanism



Sorting Tray Wells

The Autoclone Sorting Option steps through the wells of the tray in a serpentine path, skipping unprogrammed wells. For example, in a 24-well tray, the first well will be A1 followed by A2, A3, A4, A5, A6, B6, B5, B4, B3, B2, B1, C1, C2, C3, C4, C5, C6. At this point, the plate is as far to the left as possible; therefore, the Autoclone Sorting Option rotates the tray 180 degrees and then continues to sort until the tray is finished.

Preventing Electrostatic Build-Up

Electrostatic build-up in the sort wells is prevented because the Autoclone Sorting Option does not charge droplets containing target cells. The waste droplets are charged and deflected into the waste catcher; and only uncharged droplets containing the desired cells fall into the sort wells.

Mechanical Assembly Description

Motors

The assembly contains three stepper motors: two move the collection tray from left to right and from front to back, and the third motor rotates the collection tray clockwise (CW) or counterclockwise (CCW). The motors rotate a discrete number of degrees whenever the current is applied to their multiple phase windings.

The tray rotation motor is inside the arm on top of the assembly and directly rotates the collection tray holder.

Rotation of the motor shaft is monitored by two optical sensors that detect when the motor shaft is in the normal position (ready for a tray to be loaded) or in 180-degree rotated position. A third optical sensor detects the presence of the cover assembly installed by the operator when the Autoclone Sorting Option is retracted and not in use.

Tray Rotation Arm

The tray rotation arm is mounted on a translation stage that moves from left to right when driven by a stepper motor. This movement is monitored by optical sensors that move with the stage. Holes drilled in a stationary metal strip trigger one of the optical sensors at each extreme of the stages normal travel. If the stage overtravels in either direction, a mechanical switch closes alerting the electronics and the operator that an error has occurred.

The left-right stage is mounted to the forward-backward movement stage. This stage is also moved by a stepper motor and has optical sensors to determine when the stage is in the full-forward (load) position, back to the end of the tray (home) position, and the full-backward (retracted) position. The stage also carries overtravel switches to give an error condition if the stage moves too far forward or back.

Optical Sensors

You can see the status of the optical sensors on the Autoclone Sorting Option screen on the Cytometer in the box labeled Markers.

Only those sensors currently read appear on the screen. See Table 2.2-1.

Table 2.2-1 Autoclone Sorting Option Screen Marker Definitions

Markers on Screen	Description
Lx	Tray is in the full-forward (load) position. Row 12 of a 96-well plate is under the flow cell when this marker is displayed.
Hx	Tray is in the back (home) position. Row 1 of a 96-well plate is under the flow cell when this marker is displayed.
Ly	Tray is all the way to the left. Column A or column H of a 96-well plate is under the flow cell when this marker is displayed.
Hy	Tray is all the way to the right. Column D or column E is under the flow cell when this marker is displayed.
CW	Tray is in the load rotational position (the cutout is to the front).
CC	Tray is rotated 180 degrees (the cutout is in the back).
Hx and Lx both appear	Arm is in the fully back (retracted) position.
CC and CW both appear	ERROR condition; tray holder base is not in place or sensor is defective.

Autoclone Sorting Option Card

The Autoclone Sorting Option card interfaces between the Cytometer (multibus) CPU and the Autoclone Sorting Option mechanism. Through this card, the CPU operates the mechanism motors to move the tray and read the optical position sensors and limit switches. This is not an intelligent (microprocessor equipped) card. The system CPU must issue an I/O command for each step of the stepper motors. The card converts a command from the CPU into the appropriate four phase current to move one of the stepper motors.

The status of the optosensors and the limit switches are encoded and placed on the databus for the CPU to read. The status of the optosensors and limit switches are also displayed by LEDs on the card. Note that the limit switches are latched on the card. Once a limit switch has been activated, the error condition remains until the card is reset. This is done by recalibrating the Autoclone Sorting Option.

Autoclone Sorting Option Interconnect Card

The Autoclone Sorting Option Interconnect card is attached to the rear of the mechanical assembly and serves as the connection point for the cable to the Cytometer. The card contains no active components but provides connectors for the two flex cables and a connection to the X-motor (front-back).

Position Detection Cards

Two Position Detection cards are used in the mechanism: one is mounted to the X-movement stage and one to the Y-movement stage.

Each card has two optical sensors and two mechanical switches. The optical sensors detect holes punched in a fixed metal strip indicating the normal travel limits of the stage mechanism. The mechanical (micro) switches are present only to detect if accidental overtravel has occurred and to advise the operator to calibrate the system.

Software

ATTENTION: Autoclone Sorting Option software resides in both the Workstation and the Cytometer. The Workstation software obtains the necessary information from the user: the type of tray, which wells to sort into, number of cells to put into each well, and the sort criteria for each well. The Workstation transfers the information to the Cytometer through the serial communication path.

The Cytometer software physically operates the Cytometer mechanism to operate the motors and interpret information from the optical and mechanical sensors. It also supports the Autoclone Sorting Option Control and the Adjust and Retract screens on the Cytometer. Through these screens, you can initiate calibration and adjustment routines.

Calibration

The calibration routine operates each stepper motor in turn to find the limits of travel using the optical sensors and to count the number of steps between the two limits. With this information, the Cytometer computer ‘knows’ where the mechanism is and can calculate the number of steps to move each stepper to position the tray correctly. This sequence must be successfully completed before the Autoclone Sorting Option can operate. Calibration also needs to be performed if the movement of the mechanism is interfered with, a limit switch has been activated, or the movement speed has been changed.

Autoclone Sorting Option Mechanism Adjustment

The speed of movement, the step rate, of the motors must be adjusted to optimize operation of the system. The X-Y speed is adjusted to provide smooth movement of the mechanism from left to right and in and out. Rotational speed of the tray is controlled by the ROT speed. The rotation is adjusted so it is smooth and slow enough that liquid in the wells will not be spilled. Calibration must be performed after each speed change to ensure the mechanism does not overtravel and strike a limit switch.

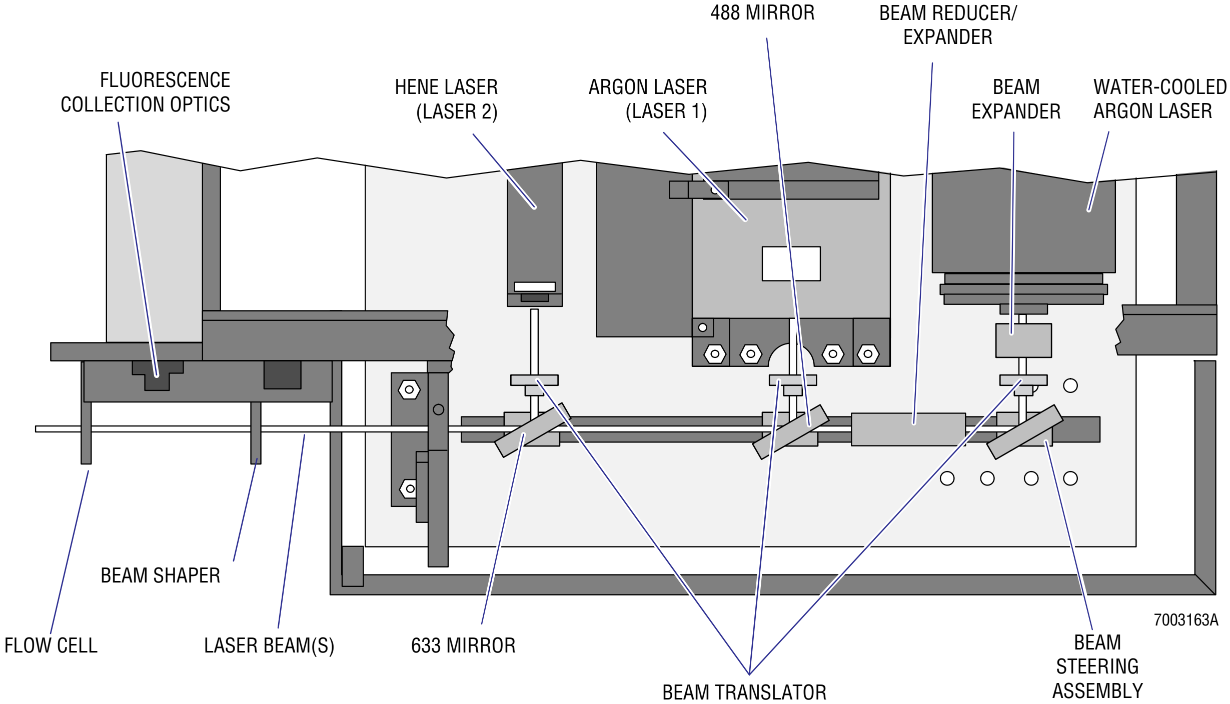
Refer to Chapter 6 of the Options Manual for instructions on how to operate the Autoclone Sorting Option.

For installation instructions, refer to Heading 3.10, AUTOCLONE SORTING OPTION INSTALLATION.

2.3 OPTICAL COMPONENTS

Figure 2.3-1 illustrates the various optical components of the Elite.

Figure 2.3-1 Optical Components, Top View with Cover Removed



Lasers

Refer to Table 2.3-1 and Table 2.3-2 for a summary of lasers, including technical specifications.

Table 2.3-1 Laser Summary

	Air-Cooled			Water-Cooled			Air-cooled
Laser	**Argon**	**HeNe**	**HeCd**	**I90**	**I305**	**Enterprise**	**Green HeNe**
Wavelengths/nm	488	633	325	1. 351-364 2. 457 3. 465 4. 472 5. 476 6. 488 7. 496 8. 501 9. 514	1. 351-364 2. 457 3. 465 4. 472 5. 476 6. 488 7. 496 8. 501 9. 514	488 351-364	543.5
Maximum Power/ mW	15	10	20	1. 200 2. 350 3. 150 4. 200 5. 600 6. 1500 7. 600 8. 400 9. 2000	1. 200 2. 350 3. 150 4. 200 5. 600 6. 1500 7. 600 8. 400 9. 2000	150 50	2.5
Tips	Quartz	Quartz	Quartz	All	All	All	Quartz
Dyes	FITC PE (RD1) PI AO ECD Thiozole Or.	APC CY5	INDO1	FITC PE (RD1) PI AO ECD INDO1 Thiozole Or.	FITC PE (RD1) PI AO ECD INDO1 Thiozole Or.	FITC PE (RD1) PI AO ECD INDO1 Thiozole Or.	PE ECD Cychrome PC5

Table 2.3-2 Laser Technical Specifications

Laser	Specifications	
Cyonics Air-Cooled Argon*†	Output power:	15 mW of 488 nm
	TEM_{00} mode purity:	>99%
	Beam diameter at $1/e^2$:	0.65 mm
	Beam divergence:	0.95 mrad
	Polarization ratio:	250:1 minimum
	Amplitude noise:	1.5% RMS (10 Hz - 2 MHz)
	Amplitude drift:	1% maximum (light control mode over 2 hours
	Warm-up time:	5 minutes maximum
	Beam pointing stability:	<±0.03 mrad after warm-up (temperature range ±3·°C)
	Lifetime:	>10,000 hours at specified power
	Operating voltage:	115 Vac ±10%
	Operating current:	17.0 A maximum, single phase
Uniphase Red HeNe†	Output power:	10 mW minimum
	Wavelength:	632.8 nm
	Polarization:	Random
	Spatial mode:	TEM_{00}
	Beam diameter at $1/e^2$:	0.68 ±0.02 mm
	Beam divergence:	1.2 mrad
	Amplitude noise:	0.5% RMS (30 Hz - 10 MHz)
	Amplitude drift:	±3%
	Warm-up time:	15 minutes maximum
	Beam pointing stability:	0.02 mrad maximum after warm-up
	Lifetime:	10,000 hours minimum
	Operating voltage:	3100 ±200 Vdc
	Operating current:	6.5 ±0.2 mA
Melles Griot Green HeNe†	Minimum power:	1.5 mW
	Wavelength:	543.5 nm
	Mode:	
	Polarization:	Random
	Beam diameter at $1/e^2$:	0.80 ±5% mm
	Beam divergence:	0.86 ±5% mrad
	Amplitude drift:	<2.5%
	Amplitude noise:	<2.8% 30 Hz-30 MHz (peak-to-peak)
	Warm-up time:	7 minutes
	Beam pointing stability:	<0.03 mrad (after 15 minutes)
	Operating temperature:	–20 to +40°C
	Expected tube life:	8,000 hours
	Line frequency:	48 - 63 Hz
	Operating voltage:	3,000 ±100 Vdc
	Operating current:	6.5 ±0.2 mA

**Due to low output power, this laser is not recommended for sense-in-air operation.*
†Air-cooled laser.
‡Water-cooled laser.

Table 2.3-2 Laser Technical Specifications *(Continued)*

Laser	Specifications	
Coherent DPSS†	Wavelength:	532 nm
	Polarization:	>1:100
	Output power:	20 mW minimum
	Spatial mode:	TEM_{00}
	Beam diameter at $1/e^2$:	0.6 mm
	Beam divergence:	≤1.3 mrad
	Amplitude noise:	<0.5% RMS (10 Hz - 10 MHz)
	Warm-up time:	<5 minutes
	Beam pointing stability:	<07.5 µrad/°C
	Operating voltage:	100/115/200 Vac ±10%
Omnichrome HeCd 74†	Minimum power when	30 mW
	shipped:	20 mW
	Warranted minimum power:	325 nm
	Wavelength:	Forced air
	Cooling:	Multi-mode
	Mode:	1.2 mm
	Beam diameter at $1/e^2$:	0.5 mrad
	Beam divergence:	<5 minutes
	Warm-up time from cold:	<2 minutes
	Recovery from standby:	10 to 40°C
	Operating temperature:	>6,000 hours
	Expected tube life:	104 - 128 Vac
	Line voltage:	48 - 63 Hz
	Line frequency:	325 nm beam splitter
	Optical filters:	325 nm long pass
		381 nm band pass
		440 nm long pass
Coherent Innova Enterprise‡	Wavelength/output power:	488 nm, 150 mW
		351/364 nm, 50 mW
	Mode:	TEM_{00}
	Polarization ratio:	100:1
	Beam diameter at $1/e^2$:	1.20 mm (488 nm), 0.88 mm (UV)
	Beam divergence:	0.82 mrad (488 nm), 0.73 mrad (UV)
	Beam pointing stability:	<5 µrads/°C
	Optical noise:	<1.0% RMS
	Power stability:	<1.0% (UV)
	Input voltage:	208 - 240 Vac ±10%, 50 or 60 Hz, single phase
	Input current:	31 A ±10%
	Cooling water:	8 - 16 liters/minute (2 - 4 gpm)
	Weight:	Laser head: 27 kg (59 lb)
	Power supply:	36 kg (78 lb)

**Due to low output power, this laser is not recommended for sense-in-air operation.*
†Air-cooled laser.
‡Water-cooled laser.

Table 2.3-2 Laser Technical Specifications *(Continued)*

Laser	Specifications	
Coherent Innova 305‡	Wavelength/output power:	528.7 nm, 0.35 W
		514.5 nm, 2.00 W
		501.7 nm, 0.40 W
		496.5 nm, 0.60 W
		488.0 nm, 1.50 W
		476.5 nm, 0.60 W
		472.7 nm, 0.20 W
		465.8 nm, 0.15 W
		457.9 nm, 0.35 W
		454.5 nm, 0.12 W
		363.8 nm, 0.14 W
		351.1 nm, 0.14 W
		333.6 to 363.8 nm, 0.40 W
	Beam diameter at $1/e^2$:	1.5 mm
	Beam divergence:	0.5 mrad
	Beam pointing stability:	<5 µrads
	Optical noise:	0.2% RMS
	Amplitude drift:	±1%
	Input voltage:	208 Vac ± 10%, 50 or 60 Hz, 3-phase
	Input current:	50 A/phase
	Cooling water:	8.5 liters/minute (2.2 gpm)
	Weight:Laser head:	42 kg (92 lb)
	Power supply:	39 kg (86 lb)
Coherent Spectrum‡	Wavelength/output power:	647.1 nm, 0.25 W
		568.2 nm, 0.15 W
		514.5 nm, 0.25 W
		488.0 nm, 0.25 W
		476.5 nm, 0.10 W
		457.9 nm, 0.03 W
	Beam diameter at $1/e^2$:	UV multiline, 0.05 W
	Beam divergence:	1.5 mm (647.1 nm)
	Optical noise:	0.8 mrad
	Input voltage:	0.5% RMS
	Input current:	208 Vac ± 10%, 50 or 60 Hz, 3-phase
	Cooling water:	40 A/phase
	Weight:	8.5 liters/minute (2.2 gpm)
	Laser head:	43 kg (95 lb)
	Power supply:	34 kg (75 lb)

**Due to low output power, this laser is not recommended for sense-in-air operation.*
†Air-cooled laser.
‡Water-cooled laser.

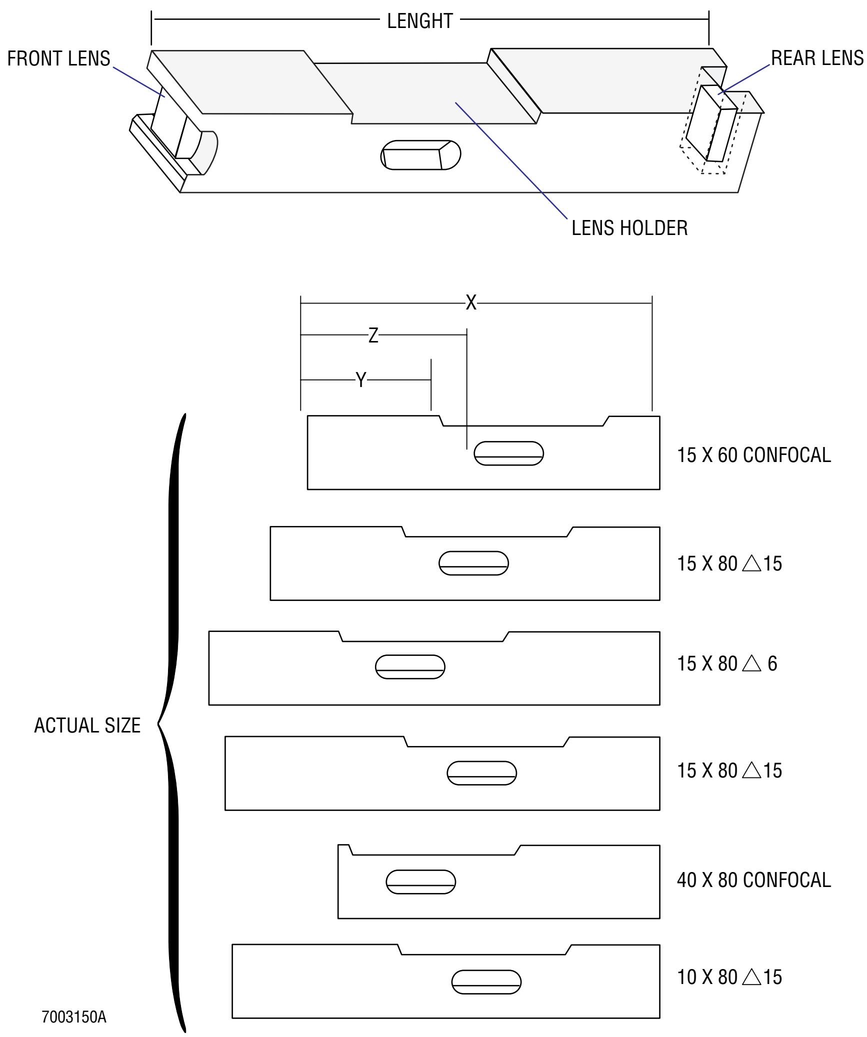
Beam Shapers

There are 7 beam shaper assemblies available (Figure 2.3-2):

- 15 x 60 confocal
- 15 x 80 Δ 15

- 15 x 80 Δ 6
- 8 x 80 Δ 15
- 40 x 80 confocal
- 10 x 80 Δ 15

Figure 2.3-2 Beam Shaper Options



Each assembly consists of a sealed pair of crossed cylindrical lenses that produce a specific elliptical beam cross-section at the flow cell. The lenses are centered in each assembly and allow the user to change beam shapers with only minor adjustments.

The front lens (nearest the flow cell) is horizontal and determines the height of the laser beam (parallel to the flow). The vertical rear lens determines the width of the laser beam (orthogonal to the flow).

Beam height determines peak signal pulse width. For doublet discrimination, cell size must be at least half the height of the beam. Beam width directly affects sensitivity and resolution.

See Heading 2.7, OPTICAL ELEMENTS for additional information on the optical element theory.

Table 2.3-3 shows approximate working beam spot dimensions obtained using various combinations of laser and focusing lens. To use the table:

- Locate the appropriate laser and wavelength in the table.
- Read the beam height and width from the appropriate columns for the focal length of the lenses in the beam shaper you are using.

For example, Coherent 305 Laser, with 488 nm, using 8 x 80 defocused 5.8 mm beam shaper, gives the following: beam height of 3.4 µm, and beam width of 112 µm.

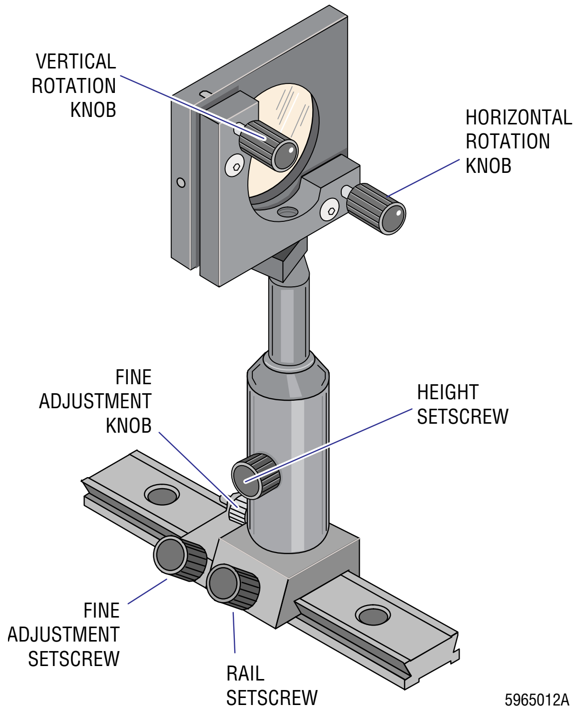
Table 2.3-3 Working Beam Spot Dimensions

Laser	Wavelength (nm)	Beam Diameter (mm)	Front Lens Focal Length (mm)				Rear Lens Focal Length (mm) and Defocus Distance (mm)			
			8	10	15	40	60	80	80 (5.8)	80 (15.1)
			Beam Height (µm)				Beam Width (µm)			
Cyonics	488	0.67	6.4	8.0	12.1	33.4	51.3	70.0	86.9	151
Uniphase	633	0.68	7.6	9.5	14.4	40.0	61.9	85.0	102	169
Melles Griot	544	0.80	6.3	7.8	11.8	32.1	48.8	67.0	89.8	172
DPSS	532	0.60	8.0	10.0	15.2	42.4	65.8	90.7	107	175
Omnichrome	325	1.30	4.1	5.1	7.7	20.6	30.9	41.2	137	343
Coherent 300 Series and Coherent Spectrum	351+365	1.24	3.6	4.5	6.8	18.2	27.4	36.6	169	432
	457	1.42	3.3	4.1	6.1	16.3	24.5	32.7	108	271
	488	1.46	3.4	4.2	6.3	16.9	25.4	33.9	112	288
	515	1.50	3.5	4.3	6.5	17.4	26.1	34.9	114	286
Spectrum only	647	1.50	6.3	7.9	11.8	31.6	47.4	63.3	171	418
Coherent Enterprise	351+365	0.67	3.4	4.2	6.4	17.4	26.6	36.1	81.5	193
	488	0.80	3.7	4.6	7.0	19.0	29.0	39.4	105	256

Beam Steering Optics

When an Elite is configured with multiple lasers, beam steering optics are used to allow the beams to be combined or precisely separated at the flow cell. Dichroic mirrors pass and/or reflect specific wavebands, the mirrors from the optical component of the beam steering assemblies. Figure 2.3-3 illustrates an alignment mirror.

Figure 2.3-3 Alignment Mirror



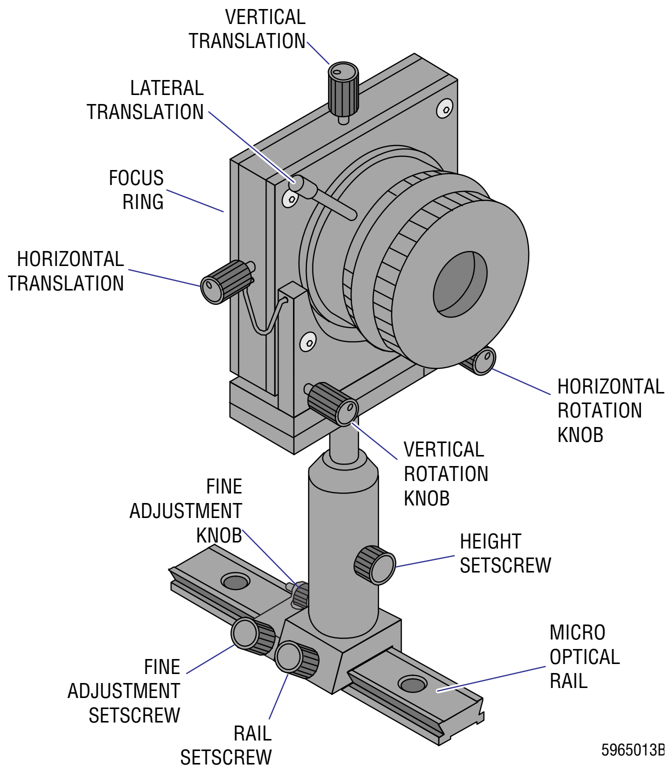
Beam Translators

A beam translator displaces the laser beam without changing its orientation. This is the same effect as moving the laser, but the beam translator simplifies the process because the movement is more controlled.

Beam Expander/Reducer

A beam expander/reducer (Figure 2.3-4) balances the characteristics of small, air-cooled lasers with those of large water-cooled or HeCd lasers. This enables both laser types to be focused at the same distance from the beam shaper. Reverse the beam expander/reducer if you want to perform the opposite function. If the expander/reducer is turned so the expander side is used, you can reverse it by turning it around to the reducer side.

Figure 2.3-4 Beam Expander/Reducer



The laser beam must pass through the optical center of the beam expander/reducer to prevent optical irregularities. The beam expander/reducer should be installed directly in front of the laser to allow the beam translator and steering optics to be adjusted without having to realign and focus the beam expander/reducer each time.

Side Scatter and Fluorescence Collection Optics

Sort Sense Flow Cell Tips

Sort sense flow cell is designed for Sense-in-Quartz sorting using an air cooled laser. It is the principal flow cell for the Elite ESP. The sort sense contains a 250 µm square flow channel and a 100 µm diameter jetting orifice. There are two designs: the original sort sense (Figure 2.3-5) and the new 3x sort sense (Figure 2.3-6).

Figure 2.3-5 Sort Sense Flow Cell, Original

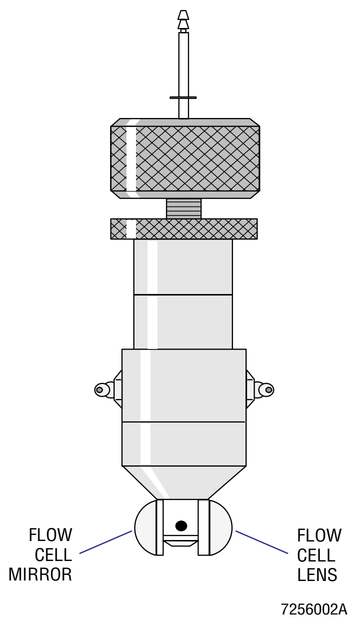
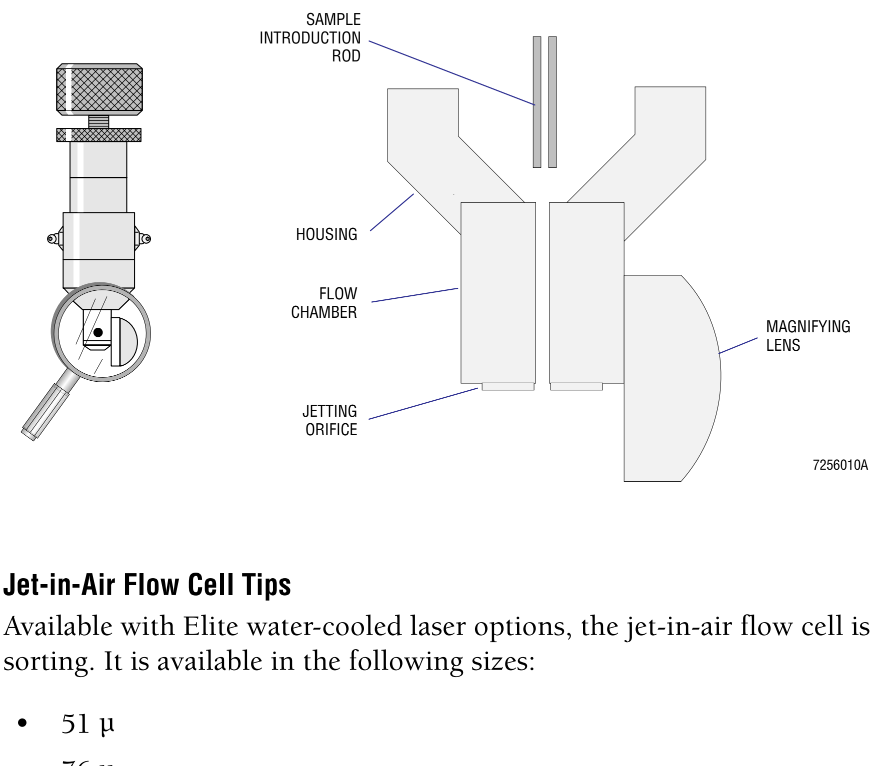


Figure 2.3-6 100 µ 3x Sort Sense Flow Cell, Cut-away View



Jet-in-Air Flow Cell Tips

Available with Elite water-cooled laser options, the jet-in-air flow cell is used for high-speed sorting. It is available in the following sizes:

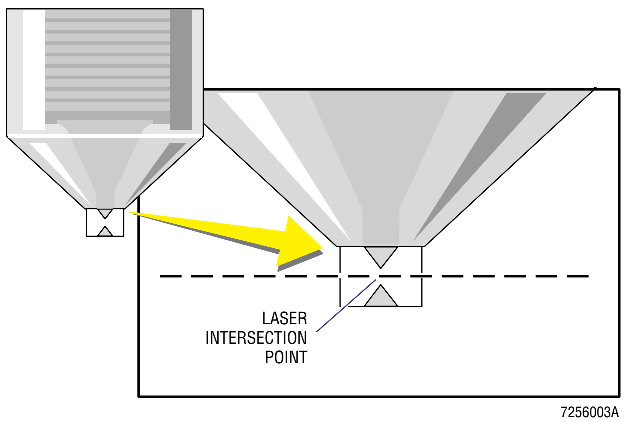
- 51 µ
- 76 µ
- 100 µ
- 150 µ
- 200 µ

When using the jet-in-air flow cell, an obscuration bar must be placed in front of the fluorescence pickup lens.

Fast Quartz Flow Cell

The 76 µ high velocity flow cell incorporates the high signal-to-noise characteristics of sense-in-quartz design of the sort sense with the sorting speed advantages of the jet-in-air design. The interrogation point is within the 76 µ cuvette, which minimizes the interference from stream perturbations. See Figure 2.3-7.

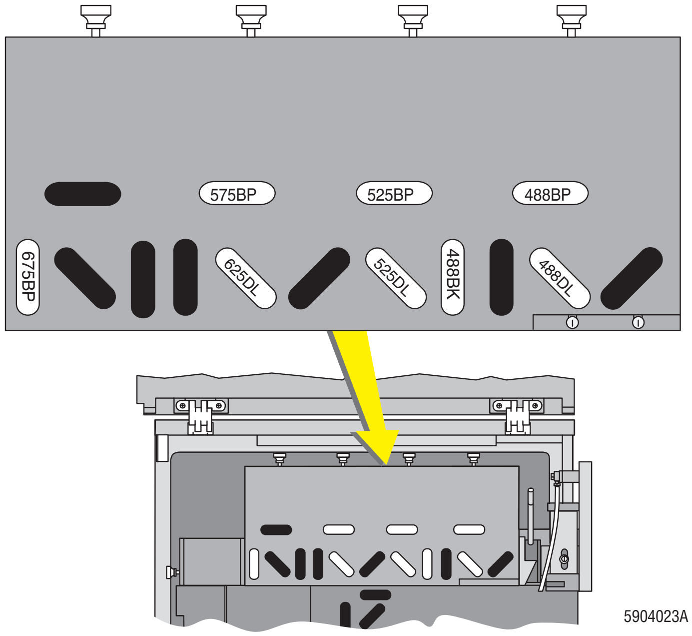
Figure 2.3-7 76 µm High Velocity Quartz Flow Cell



Optical Filters

The Elite uses optical filtering to select bandwidths corresponding to specific fluorochromes. The optical block allows you to view the filter configuration at a glance and easily change the filter configuration. See Figure 2.3-8.

Figure 2.3-8 Optical Filters, Location



Each filter holder is clearly labeled to identify the filter and can be read when the holder is installed in the filter block.

Long Pass (LP) Filters

LP filters absorb short wavelength light and pass long wavelength light. They are identified by their 50% transmittance wavelengths. Do not use these filters as the only means to block light.

Short Pass (SP) Filters

SP filters pass short wavelength light and reflect long wavelength light. They are identified by their 50% transmittance wavelength.

Blocking (BK) Filters

BK filters pass light at all wavelengths except for a narrow band of blocked wavelengths. The filters are identified by the laser line they most effectively block; they typically pass about 0.01 to 1.0% of those wavelengths. Use BK filters in the detector compartment to block intense laser light scatter.

Band Pass (BP) Filters

BP filters pass a narrow band of wavelengths and block all others. Use these filters to pass fluorescence light from a single dye, while blocking light from other dyes.

Dichroic Long Pass (DL) Filters

DL filters pass longer wavelengths and reflect shorter ones. These filters are identified by their 50% transmittance wavelength. Use these filters in diagonal positions.

Dichroic Short Pass (DS) Filters

DS filters pass shorter wavelengths and reflect longer ones. DS filters are not available through Beckman Coulter.

Neutral Density (ND) Filters

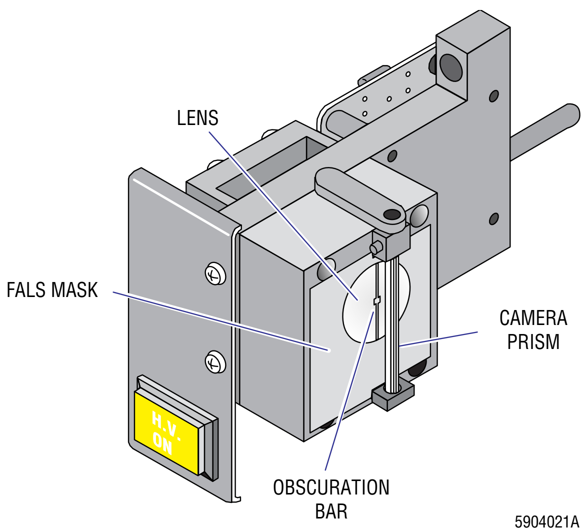
ND filters do not discriminate light by wavelength; they block all wavelengths equally. Use ND filters in front of the forward scatter detector to prevent detector saturation.

Forward Scatter Detector

The forward scatter lens collects light that is scattered between 1.4 degrees and 19 degrees from the axis of the laser beam.

The forward scatter light is extremely bright. A neutral density filter must be between the lens and the photovoltaic cells to reduce the intensity. A bar on the FALS MASK in front of the detector blocks the laser beam. See Figure 2.3-9.

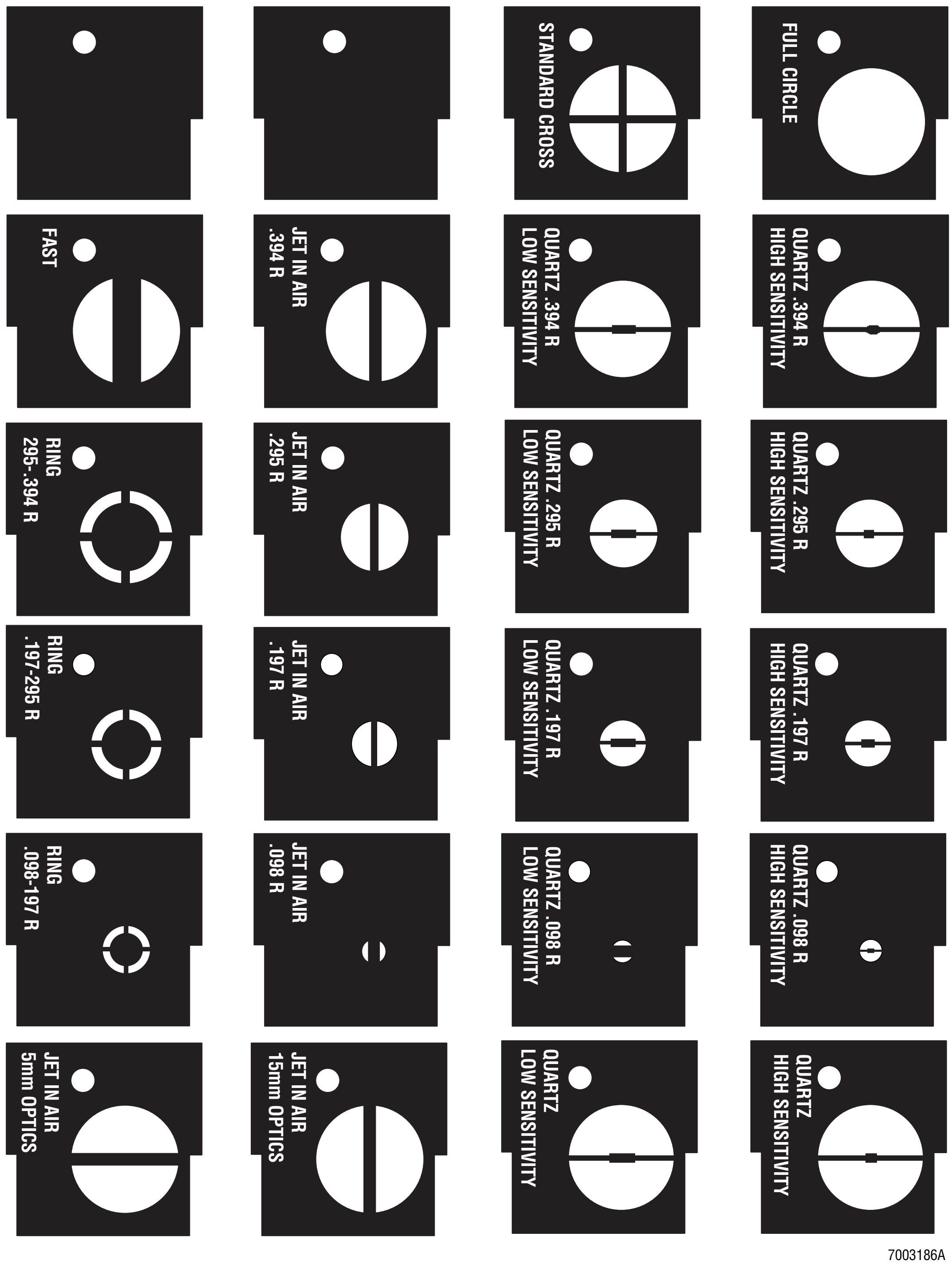
Figure 2.3-9 Forward Scatter Detector



Forward Scatter Obscuration Templates

Different applications may require different obscuration templates. Figure 2.3-10 shows the available templates.

Figure 2.3-10 Forward Scatter Obscuration Templates



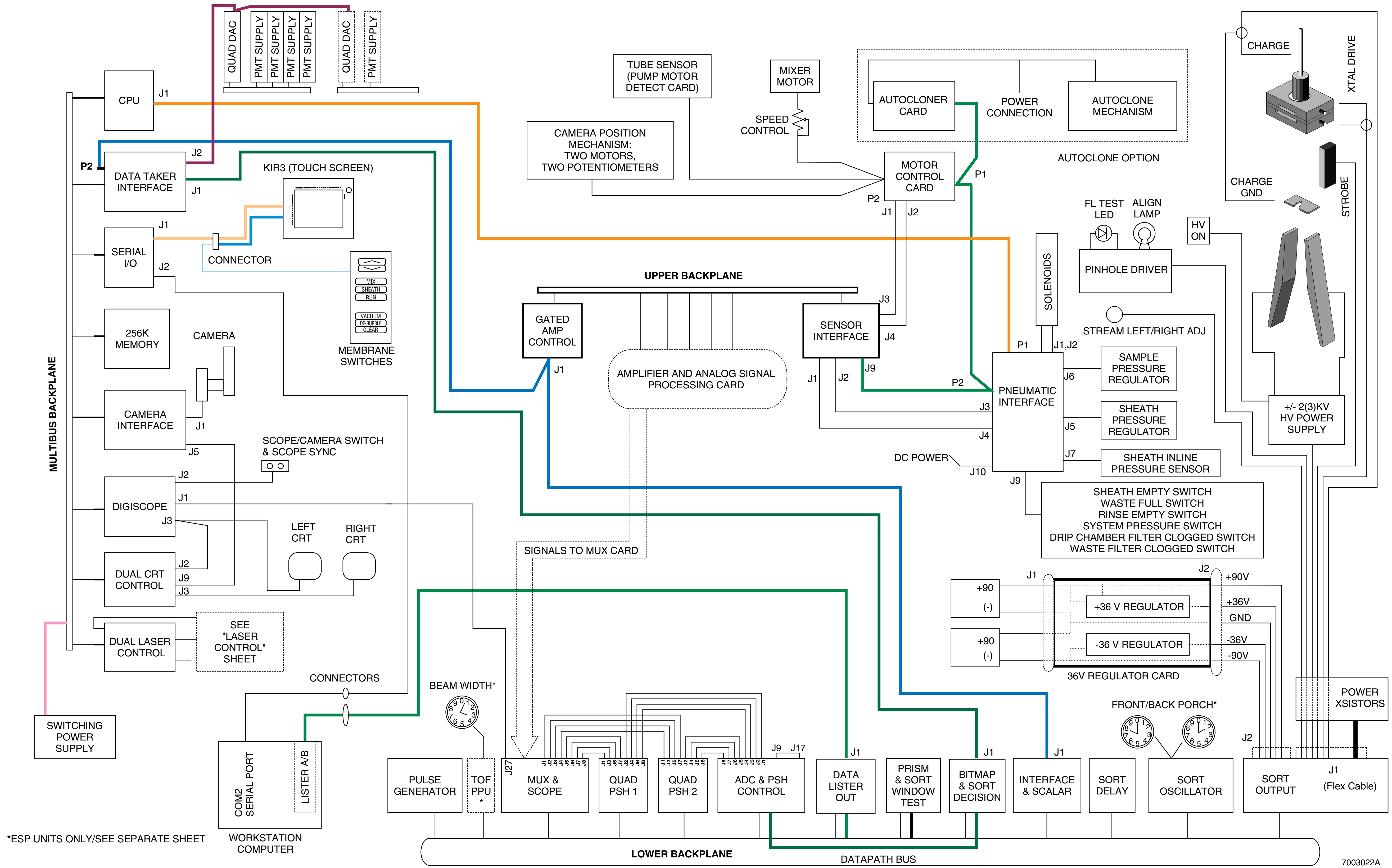
2.4 ELECTRONICS SUBSYSTEM

Description

Figure 2.7-1 is a block diagram of the Elite. The left section consists of the Multibus card cage and peripheral devices. Refer to Heading 2.5, CONTROL AND DISPLAY SUBSYSTEMS for more information. The upper and lower card cages contain cards to support data acquisition, sorting, and fluidic control functions. Refer to the appropriately named subsystem sections of this manual for more information.

Note: The configuration of the upper backplane varies with the configuration of the instrument. Refer to Heading 2.6, ACQUISITION SUBSYSTEM for more information.

Figure 2.4-1 Block Diagram, System Electronics



2.4-4

2.5 CONTROL AND DISPLAY SUBSYSTEMS

Multibus Subsystem

The Multibus subsystem is the control computer for the Cytometer. The Multibus is an industry standard backplane that provides the necessary:

- Address lines
- Data lines
- Timing lines
- Control lines.

These lines allow the various cards attached to the multibus to function as a computer system. Although many lines, such as address and data, are common to all card slots, there is a set of priority lines that are not. This means that the CPU card must always reside in slot 1 (top), but nonprocessing cards will work in any available slot.

The Multibus subsystem consists of the:

- Multibus CPU card
- External Memory (or 256K Memory) card
- Data Taker Interface card
- Serial I/O card
- Camera Interface card
- Digiscope card
- Dual CRT Control card
- Dual Laser Control card.

These cards are grouped by subsystem and are discussed in Heading 2.9.

Cytometer Computer Subsystem

This subsystem consists of the:

- Multibus CPU card
- External Memory (or 256K Memory) card
- Serial I/O card.

Multibus CPU

The Multibus CPU card:

- Executes the program stored in the EPROM and the battery-backed RAM that are on the CPU and the External Memory (or 256K Memory).
- Provides a user interface with the CRT as output.
- Accepts input from the touch screen and membrane pneumatic panel.
- Communicates with the Workstation through one of the RS232 ports on the Serial Interface card.

- Controls the air cooled lasers directly through the Multibus and the Dual Laser Control card.
- Controls the two CRT displays through the Digiscope, Dual CRT Control, and Camera Interface cards. (See Video Subsystem, Cytometer for more information on these cards.)
- Connects to the Pneumatic Interface card through a parallel port. (The solenoids are controlled through this pathway.)
- Controls the remaining cards in the Cytometer through the Data Taker Interface card. See Figure 2.5-3 for details on control pathways.

External Memory (or 256K Memory) Card

The External Memory (or 256K Memory) card provides additional memory (RAM) for the Cytometer program to run.

When the system is powered off or when the External Memory (or 256K Memory) card is removed from the unit, the RAM is powered by lithium batteries. The RAM draws 250 microamps of current from the batteries. At this rate, the life of the batteries is the same as their shelf-life, five years.

Serial I/O Card

The Serial I/O card is a five-channel RS232 interface card that allows:

- The CPU to communicate with the KIR3 (touch screen) card and the Indicator card (the Sample Station control panel) through one channel.
- For communication with the Workstation through another channel.

Laser Control Subsystem

The Laser Control subsystem (Figure 2.5-1) consists of the:

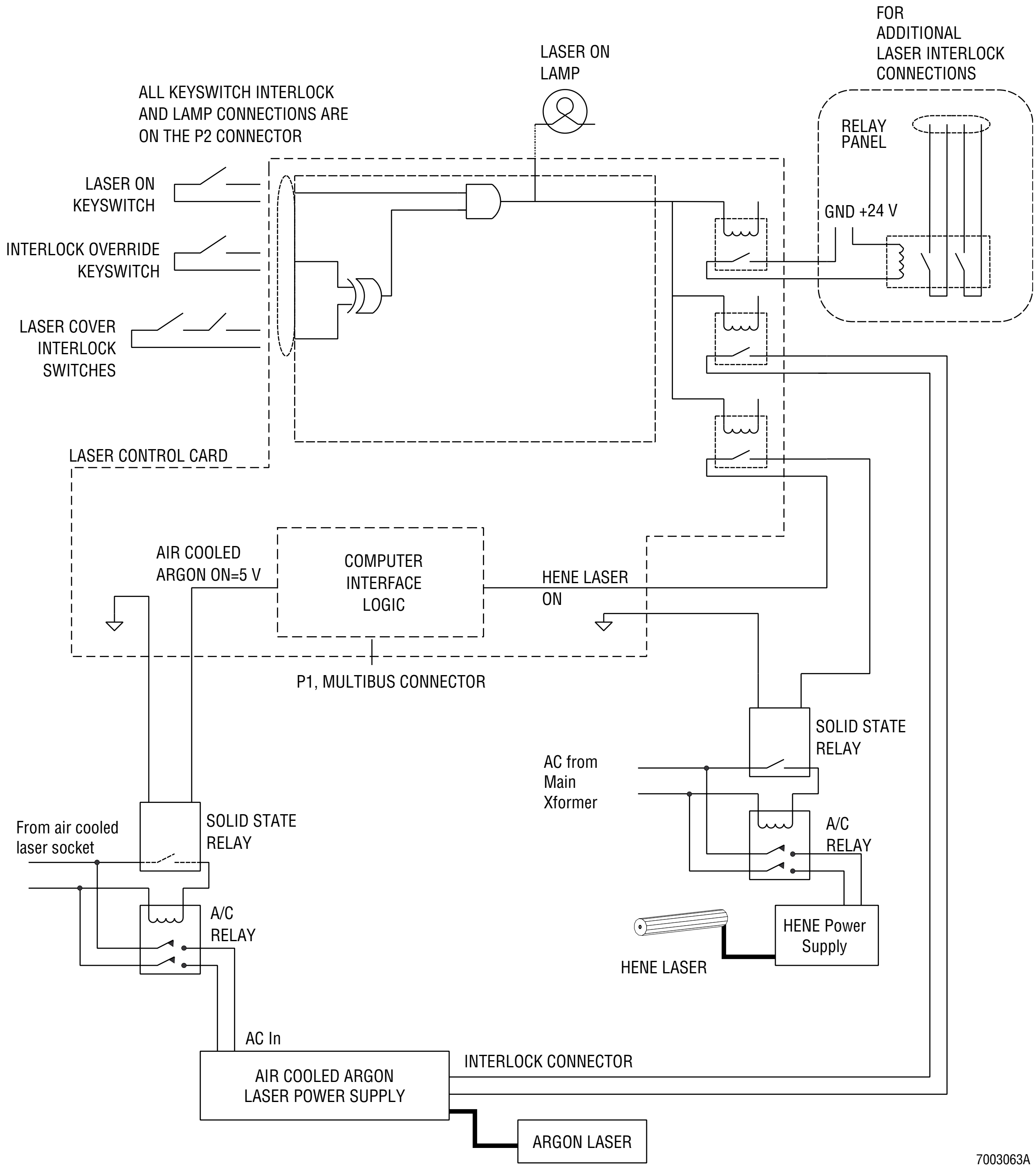
- Dual Laser Control card
- Laser relays (located in the transformer drawer)
- Interlock switches
- Relay panel (on systems equipped for additional lasers)
- Interlock keyswitches.

Dual Laser Control Card

The Dual Laser Control card has two main functions:

- The interlock function, which allows lasers to turn on only if the interlock switches are correctly configured; this function does not involve the CPU card.
- Laser control, which is controlled by the CPU card. The CPU card commands the Dual Laser Control card to turn on the laser.

Figure 2.5-1 Laser Control Subsystem



Video Subsystem, Cytometer

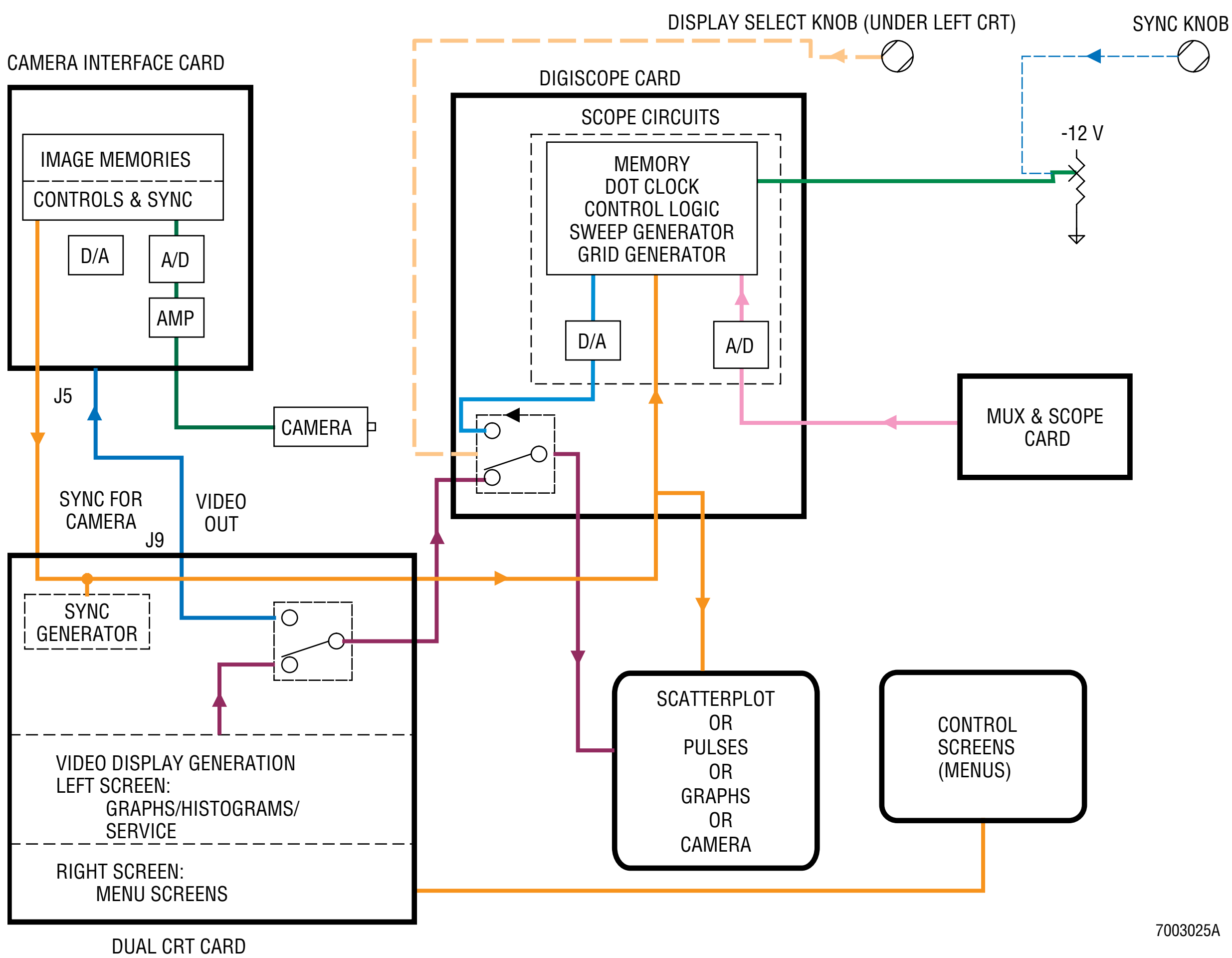
This subsystem produces the displays on the two CRT screens indicated in Table 2.5-1.

Table 2.5-1 CRT Display

CRT	Display	Source
Right	Control screens (menus)	Cytometer CPU card
Left	Pulses	Mux and Scope card
	Scatterplot	Mux and Scope card
	Graphs	Cytometer CPU card
	Camera	Camera

Figure 2.5-2 shows the pathways for the various display sources as they are processed and selected for display.

Figure 2.5-2 Block Diagram, Video Subsystem



Camera Image Signal Flow Video Display

The camera video output goes directly to the Camera Interface card via a DIN cable. The synchronization signals (horizontal and vertical “sync”) for the camera are generated on the Dual CRT Control card and are supplied to the camera through the Camera Interface card.

Camera Interface Card

The video image that the camera receives is digitized and temporarily stored in high speed memory on the Camera Interface card. This allows the Cytometer’s CPU to manipulate the camera image. The pan feature is implemented by selecting the portion of video memory to display. The sorting cursors are added to the image. The processed camera image is read out from the video memory, reconverted to an analog video signal, and routed to the Dual CRT Control card.

Dual CRT Control Card

The camera video signal passes through this card only if requested by the CPU. To do this, select **DISPLAY CAMERA** on the Cytometer Control Screen. If you do not select **DISPLAY CAMERA**, then the camera video ends here, and the card outputs the alternate video display (small histograms or service displays). The Dual CRT Control card generates the menus and routes them to the right CRT for display.

Digiscope Card

When you turn the display select knob to the camera position, this card switches the camera image (or alternate Dual CRT Control output). In the other position, the digiscope generated image is displayed.

Pulse Video Display

The Pulse Video Display consists of the:

- Mux and Scope card - This card selects the upper and lower trace signals.
- Digiscope card - This card digitizes the selected pulses and stores them in onboard memory. Logic and timing circuits use stored pulse data to provide a scan or raster-compatible video signal. The card generates the grid image that serves as a background for the pulses. The card outputs the pulse image if the Display Select knob is in the scope position. The video signal is routed to the left CRT.
- KIR3 card and membrane panel - The KIR3 card surrounds the front of the right CRT. An array of infrared-emitting LEDs and phototransistors is mounted on the card. They are aimed at each other to form a grid of horizontal and vertical light beams in front of the CRT face. A circuit on the card scans the photo transistor outputs to detect when a pair of the horizontal and vertical beams has been blocked. This information in encoded and sent to the Serial I/O card to inform the Multibus CPU card where you have placed your finger on the screen.

The KIR3 card also receives signals from the membrane panel indicating which membrane button you pushed. This information is also encoded and sent to the Serial I/O card. A dedicated microcomputer, with RAM and ROM on the chip, controls the operation of the KIR3 card.

The KIR3 card also has a piezo “beeper” that is used by the Multibus CPU card to signal the operator of error conditions and to provide audible feedback when a membrane button is pushed.

Cytometer Control Subsystem

Figure 2.5-3 shows the Cytometer control paths of the Elite. The Cytometer Control Subsystem consists of the:

- Data Taker Interface card
- Gated Amp Control card
- Sensor I/O card
- Motor Control card
- DAC card.

Description

Most of the Cytometer's circuit cards have multiple functions and settings that must be configured by the Multibus CPU card.

The CPU places an address and a data word on the multibus backplane. Each circuit card recognizes one or more addresses and responds by reading the associated data word. The data word controls a particular function on the card, with each circuit card representing one or more ports to the CPU.

The implementation of the functions is more complex, however. Although the cards on the multibus decode the complete address, cards in the upper and lower card cages are addressed less directly. The Data Taker Interface card recognizes the range of addresses corresponding to the upper and lower card racks. The system then routes the partially decoded addresses to the:

- Gated Amp Control card, which recognizes the addresses unique to the upper backplane.
- Interface and Scaler card, which recognizes the addresses unique to the lower backplane.

Data Taker Interface Card

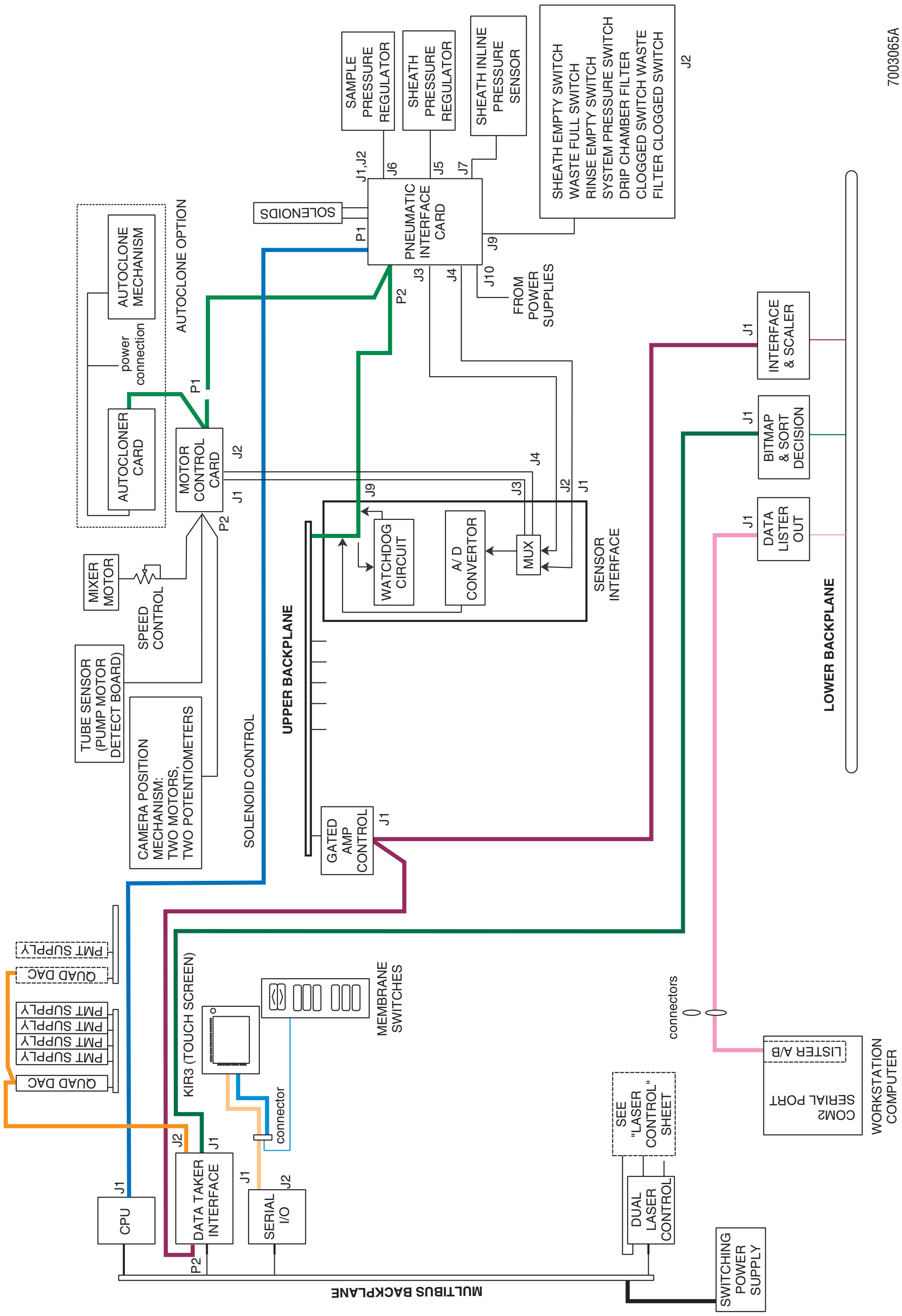
The Data Taker Interface card:

- Connects the Multibus subsystem to the three card racks in the right pedestal. The upper card rack is the Gated Amp card cage. The lower card rack is the Acquisition card cage. The racks in back of the unit are the PMT power supply card cages.
- Provides the CPU with the connection to the Gated Amp Control card to control all cards in the Gated Amp card cage.
- Provides the CPU with the connection to the Sensor Interface card to send commands to the Pneumatic Interface card and the Motor Control card.
- Provides the CPU with the connection to pass commands through the Interface and Scaler card to control all cards in the Acquisition card cage.
- Connects the Bitmap and Decision card via a ribbon cable to load the sorting bitmaps.

Gated Amp Control Card

All Elite systems have this card; it provides an interface to the gated amp backplane (upper backplane).

Figure 2.5-3 Control Pathways



Sensor I/O Card

The Sensor I/O card:

- Provides an interface between the upper backplane and the Motor Control card and the Autoclone Sorting Option Control card (with the Autoclone Sorting Option).
- Has an analog-to-digital converter that is fed by a four-channel multiplexer. Two of the channels read voltages that represent sheath and sample pressure from the Pneumatic Interface card. The other two channels are connected to the Motor Control card but are not used.
- Contains the Watchdog circuit, which is a protective circuit that monitors Multibus CPU card activity. The circuit is a timer that must be periodically reset by the Multibus CPU card. If the Multibus CPU card fails to reset the time, the Watchdog circuit sends an error signal to the Pneumatics Control card and the Motor Control card. These cards respond to this error by blocking the 24 V from the Camera Motor Control circuit, the solenoid drive circuits, and the pressure regulator drive circuits. This action prevents damage to the system in the event of Multibus CPU card failure.

Motor Control Card

The Motor Control card:

- Controls the camera's movement and zoom motors. The Multibus CPU card programs special dc motor control integrated circuits on this card for the desired position or zoom. These circuits then activate the motors until the correct feedback voltage is obtained from the potentiometers.
- Provides power to the mixer motor for fast and slow speeds. The speed control potentiometer on the front of the unit is wired in series with the mixer motor.
- Reads the tube sensor mechanism in the sample station.

DAC Card

The DAC card receives the desired PMT voltages from the Data Taker Interface card. Next, the DAC card converts the digital values to analog voltages and sends them to the proper PMT power supply (Bertans). The Bertans power supply produces a voltage output of up to 2,000 V.

2.6 ACQUISITION SUBSYSTEM

Analog Signal Processing

The sensors, PMTs, and scatter detector generate signals that are processed to develop the signals that the Mux and Scope card selects for display, acquisition, and/or sorting.

There are various configurations for this part of the system:

- 4 PMT, Nonswitchable Amps without Peak Scatter, No Gated Amp
- 4 PMT, Switchable Amp with Peak Scatter, No Gated Amp
- 5 PMT, Nonswitchable Amps without Peak Scatter, No Gated Amp
- 5 PMT, Switchable Amps with Peak Scatter, Gated Amp.

For information on the signal flow for each configuration, see Signal Flow for Each Configuration in this section.

All the configurations:

- Develop integral signals.
- Provide programmed linear gain of peak and integral signals.
- Develop logarithmically amplified signals.
- Provide subtraction (FL compensation).

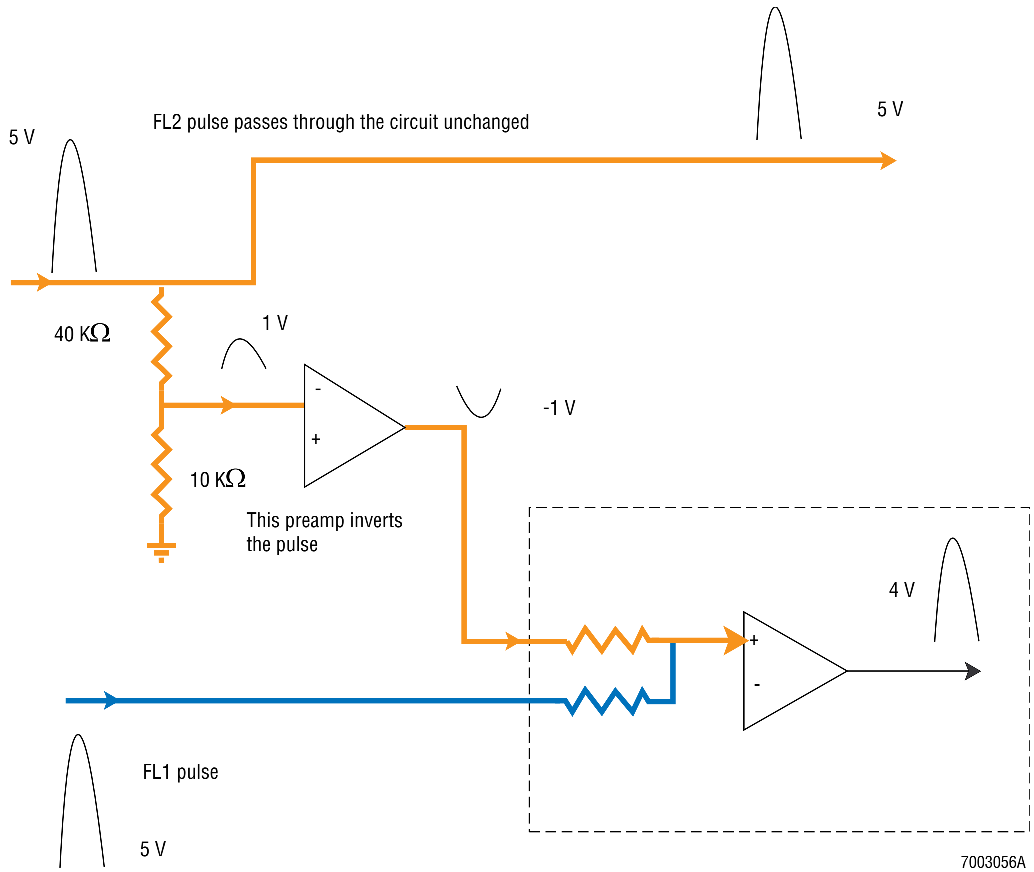
Subtraction (Color Compensation)

Color compensation allows the use of dyes that have overlapping emission spectra. Electronic subtraction circuits are used to perform color compensation.

Figure 2.6-1 shows the operation of a single subtraction circuit:

- FL2 is divided by a resistor network to provide the desired subtraction percent. The values shown in Figure 2.6-1 are for 20% (1/5). In the actual circuit, an electronically controlled divider, called a DAC, is used.
- The circuit is configured to add two voltages.
- The output is FL1 - 20%, FL2 = 4.

Figure 2.6-1 Subtraction Circuit Example



Multiple circuits perform the various subtraction combinations required. You can verify the electronic operation of the subtraction circuit by providing the same input to the main channel and the channel to subtract. The main channel is reduced by the percent of signal selected for subtraction.

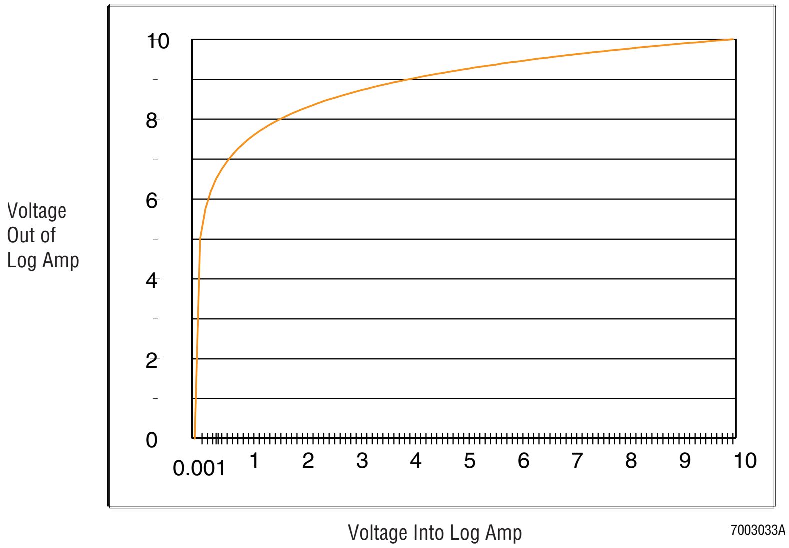
Note: The Elite performs the subtraction on peak signals. This means that the subtraction percentages directly correlate with peak signal channel numbers, but do not correspond for integral or log.

Log Amplification

The Elite uses log amplification to compress very wide distributions of information to fit conveniently on a histogram display.

Using four decade amplifiers, the Elite can accommodate a 10,000:1 range of intensity between the first and last channels of a histogram. Figure 2.6-2 shows the response of the log amp to the input voltage.

Figure 2.6-2 Log Amp Response



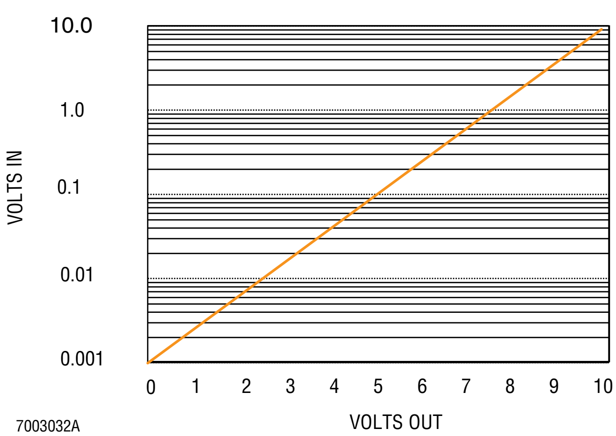
In Figure 2.6-2, the output is 0 V for an input of 1 mV (0.001 V). This corresponds to channel 0 on the histogram. Any input voltage less than this, including 0 V, results in a negative output from the log amp. Using the following formula, the amp output would approach a very large negative number as the voltage approaches 0. The Elite's circuitry includes a clamping diode that prevents the output from going below approximately 300 mV. The net result is that any signals smaller than 0.001 V will be put in channel 0.

The log amp equation is:

$$\text{Output Voltage} = \frac{10}{4} \times \log_{10}(1000 \times \text{Input Voltage})$$

Figure 2.6-3 is another graph of the log amp response. The graph is now plotted on log-lin axes. The illustration shows the four decades (10:1 ratios) covered by the log amp.

Figure 2.6-3 Log Amp Response, Log-Lin Axes



The circuit is based on the non-linear portion of a diode's V-I curve. The circuitry includes local voltage regulators and temperature compensation for stability. These circuits are adjusted at the factory with specially designed test fixtures. You cannot adjust the log amps in the field.

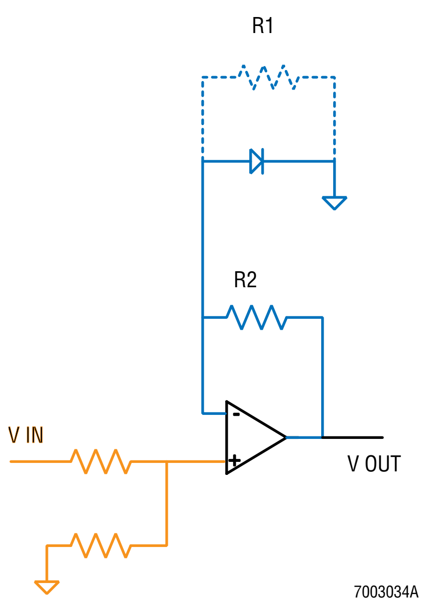
In Figure 2.6-4, operation of the circuit is based on two principles:

- The gain is (R1+R2)/R1.
- Resistor R2 is fixed, but R1 is the resistance of the diode.

Circuit elements not shown in the illustration ensure that the diode is biased to operate in the non-linear portion of its V-I curve. Based on this, for small input pulses, the diode has a low resistance and the circuit gain is high. As the input level increases, the diode's resistance increases and the circuit gain drops.

The actual circuit is considerably more complex to ensure that the diode is correctly biased and to provide precise temperature compensation.

Figure 2.6-4 Circuit Operation



Integration

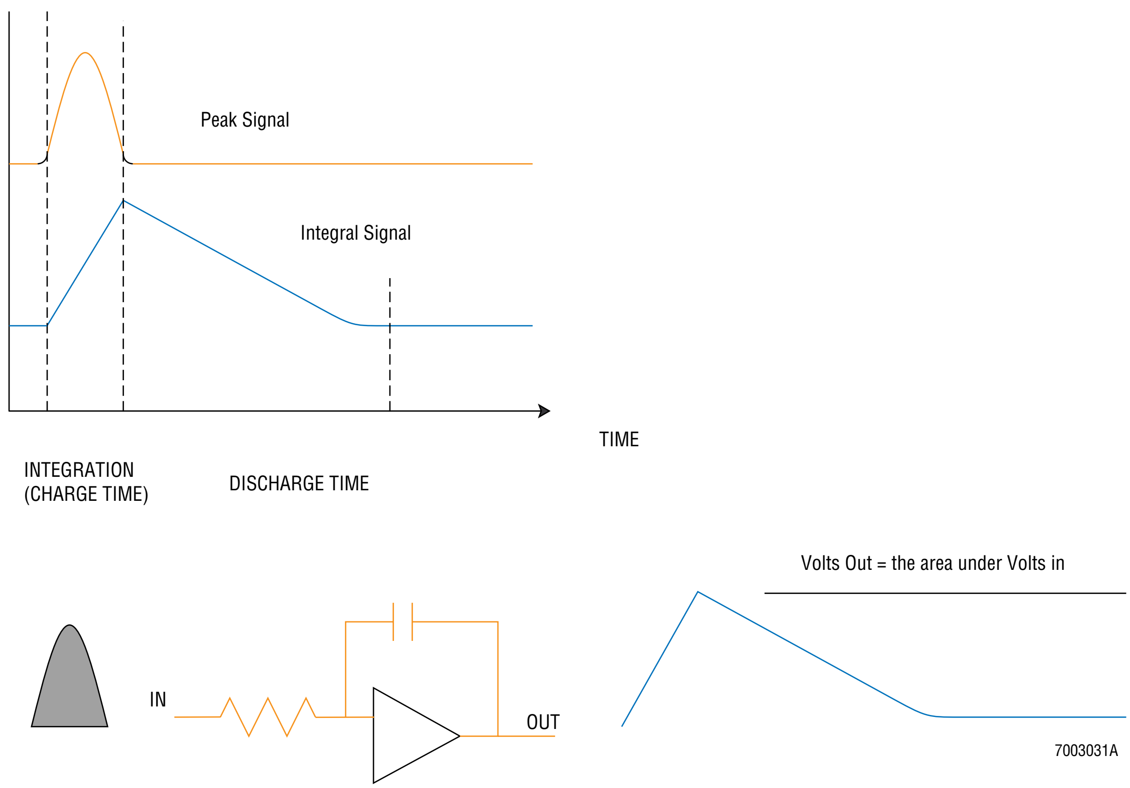
The Elite uses a standard operational amplifier integrator circuit to measure the sum of all the fluorescence in the cells. The circuit uses a capacitor in the op amp feedback to sum the input voltage over time. This results is an output voltage proportional to the area under the input pulse.

The component values of the circuit determine the length of time that the integrator can charge:

- 20 microseconds for quartz flow cells
- 5 microseconds for jet-in-air flow cells.

Once the input pulse passes, the integrator discharges. The discharge provides the characteristic tail to the integral signals. See Figure 2.6-5.

Figure 2.6-5 Integrator Discharge



Special Functions

Switchable Amplifiers (Bandwidth)

The component values of the integrator circuits must match the expected input pulse width. Depending upon the flow cell tip, the pulse width varies. The pulse width is:

- 20 microseconds for the sort sense flow cell (76 or 100 µ tip)
- 5 microseconds for the jet-in-air flow cell tip.

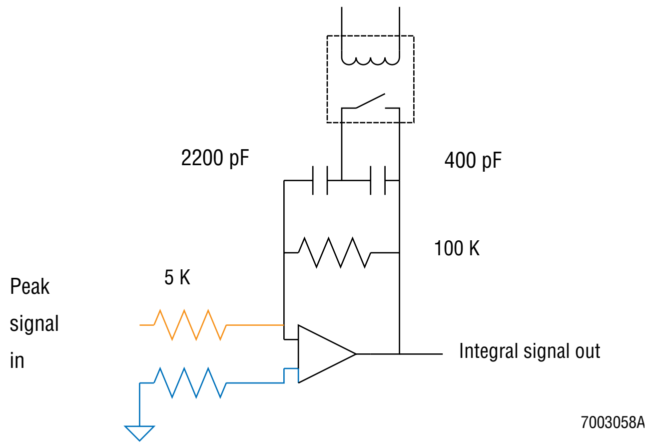
The integrators have two sets of integration capacitors to compensate for varying pulse widths. One capacitor is for quartz flow cell operation, low bandwidth, and the other capacitor is for jet-in-air flow cell operation, high bandwidth.

For all circuit cards with SWITCH or SW in their name, the CPU sends a control code to the card that activates relays on the card. This switches the correct capacitors into the appropriate circuits.

The older circuit cards in the Non-Gated Amp systems do not have the SWITCH (SW) designator. The jumpers on the card select the correct bandwidth.

Figure 2.6-6 shows the typical Switch Amp Integrator circuit.

Figure 2.6-6 Typical Switch Amp Integrator Circuit



Gated Amp Concept

A Gated Amp separates fluorescent signals that are generated as a result of excitation by two different laser beams where the respective emission spectra overlap and the wavelength (optical) filters and subtraction are not effective.

Figure 2.6-7 shows the concept of the Gated Amp using a typical application as an example, with a group of cells labeled with two different dyes: Propidium Iodide (PI) and Hoechst. Table 2.6-1 lists the emissions and requirements for each dye.

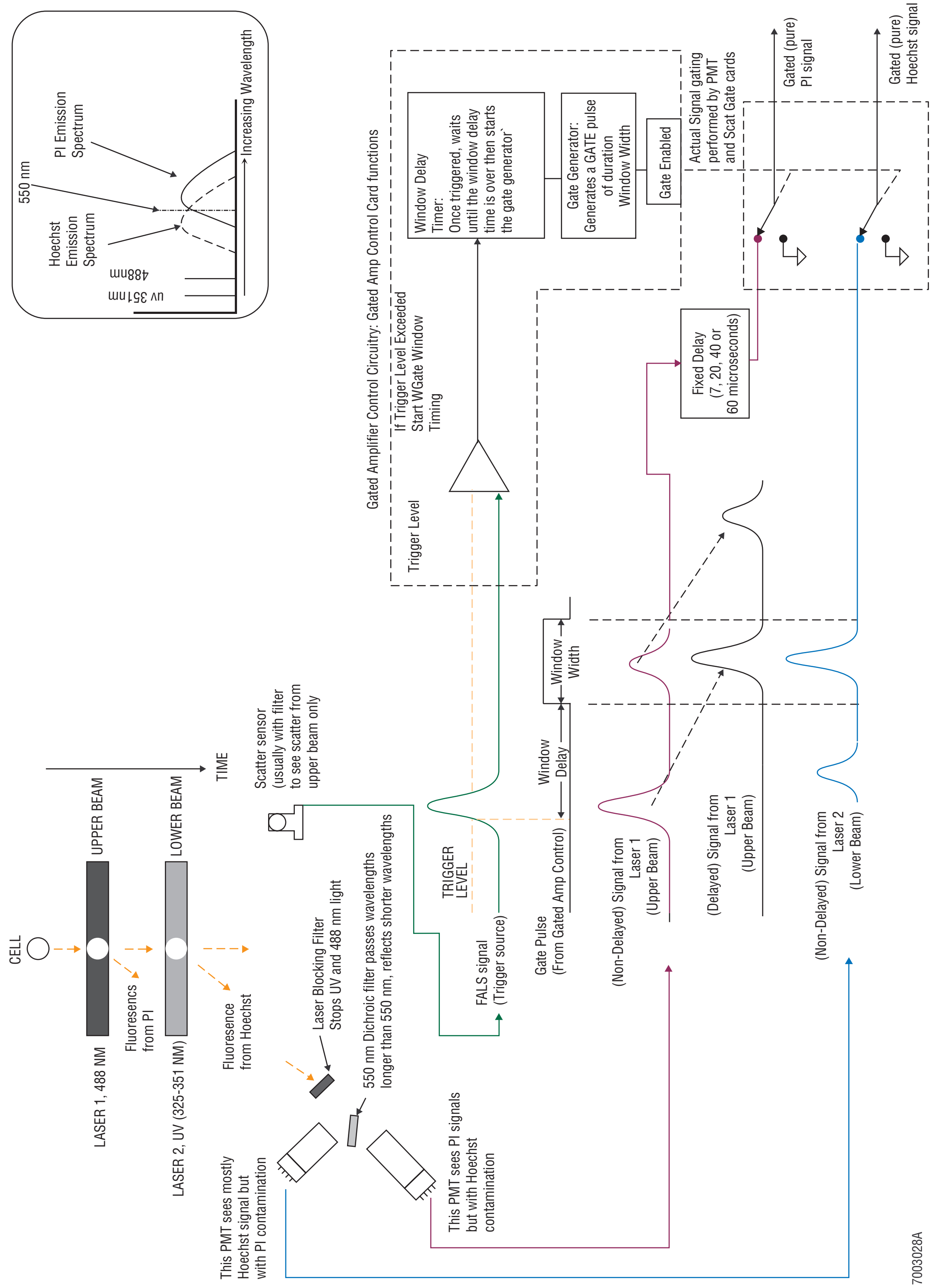
Table 2.6-1 Dye Emissions

Dye	Light Emitted	Laser Needed
Propidium Iodide (PI)	Range of wavelengths with a maximum of about 620 nm	Air-cooled Argon Laser that provides a wavelength of 488 nm
Hoechst	Range of wavelengths that overlap the PI emission band	UV Laser that provides a wavelength of 351 nm

Within Figure 2.6-7, the following are true:

- The optics are aligned so that the two laser beams intersect the sheath stream.
- The 488 beam (upper beam) is above the 351 beam.
- The 351 beam (lower beam) is below the 488 beam.
- Two PMTs and a 550 dichroic filter detect the signals.

Figure 2.6-7 Gated Amp Concept



Because of the overlap of the emission bands, both PMTs see the emissions from both dyes. In most cases, the filters and subtraction (color compensation) cannot compensate for the overlap. The Gated Amp solves the problem.

The following sequence provides a summary of what happens when a single cell passes through the laser beam:

1. As the cell passes through the upper beam, the PI dye emits light according to how much is present in the cell.
2. As the cell passes through the lower beam, the Hoechst dye emits light according to the concentration of the dye in the cell.
3. A delay line retards the signal from the upper beam by a time equal to the time required for the cell to fall from the upper to the lower beam. This occurs because the acquisition electronics expect all signal for a particular cell to be synchronous.

   The delay is 7 microseconds for a sense-in-air flow cell. This corresponds to a beam separation of 70 µ because the sheath flow outside the aperture is 10 meters/second.

   The flow rate inside the Sort-Sense flow cell is much less than that outside the aperture. Therefore, the beam separation determines which delay line is used, the 20, 40, or 60 microseconds.

The Gated Amp:

- Detects the cells by comparing the selected trigger source signal (usually FALS) to a trigger discriminator level.
- Delays all signals generated by the upper beam.
- Allows the delayed and non-delayed signals to pass on to the amplifier cards as the cell passes through the lower beam.

Refer to the individual card descriptions for information on where and how the Gated Amp functions are implemented.

Signal Flow for Each Configuration

Table 2.6-2 summarizes the Elite configurations regarding signal flow.

Table 2.6-2 Configuration Summary

Generation	4 PMT, Nonswitchable Amps	5 PMT, Nonswitchable Amps	4 PMT, Switchable Amps, with Peak Scatter Function †
	Earliest configuration.	First field-upgradable configuration.	Factory configuration available from February 1992
3 PMT Sub SW-R1 card	Compensates the FL1, FL2, and FL3 PMT signals.	Compensates combinations of the FL1, FL2, and FL3 PMT signals.*	Compensates the FL1, FL2, and FL3 PMT signals.
3 PMT Sub SW-R2 card	Not present.	Compensates combinations of the FL2, FL3, and FL4 PMT signals.*	Not present.

** The system cannot compensate for an F1 and F4 combination because they are not routed to a common card. This is not a problem, however, because the two PMTs normally use optical filters with passbands that are far enough apart to prevent any spectral overlap.*

† The peak scatter sensor is the FALS sensor in this system, and is also used in the Gated Amp system.

Table 2.6-2 Configuration Summary *(Continued)*

FL1	Routed to the Dual FL Amp card for linear and log amplification.	Routed to 3 PMT Sub SW-R1 card for compensation.	Routed to the Dual FL Amp card for linear and log amplification.
FL2	Routed to the Dual FL Amp card for linear and log amplification.	Routed to both 3 PMT Sub SW-R cards for compensation.	Routed to the Dual FL Amp card for linear and log amplification.
FL3	Completed on 3 PMT Sub SW-R card.	Routed to both 3 PMT Sub SW-R cards for compensation	Completed on 3 PMT Sub SW-R card.
FL4	Not present.	Routed to 3 PMT Sub SW-R2 card for compensation.	Not present.
Scat/CV Amp card	Processes FALS and 90LS signals.		Processes the signal from J12 to provide the integral and log FALS signals.
Mux and Scope card	Receives all processed signals.		Receives all processed signals.
Peak Scatter/Mux SW-R card	Not present.	Not present.	Receives FALS signal. J11 branches and is routed off card at J12. Processes FALS peak signal and routes to Mux and Scope card.

** The system cannot compensate for an F1 and F4 combination because they are not routed to a common card. This is not a problem, however, because the two PMTs normally use optical filters with passbands that are far enough apart to prevent any spectral overlap.*

† The peak scatter sensor is the FALS sensor in this system, and is also used in the Gated Amp system.

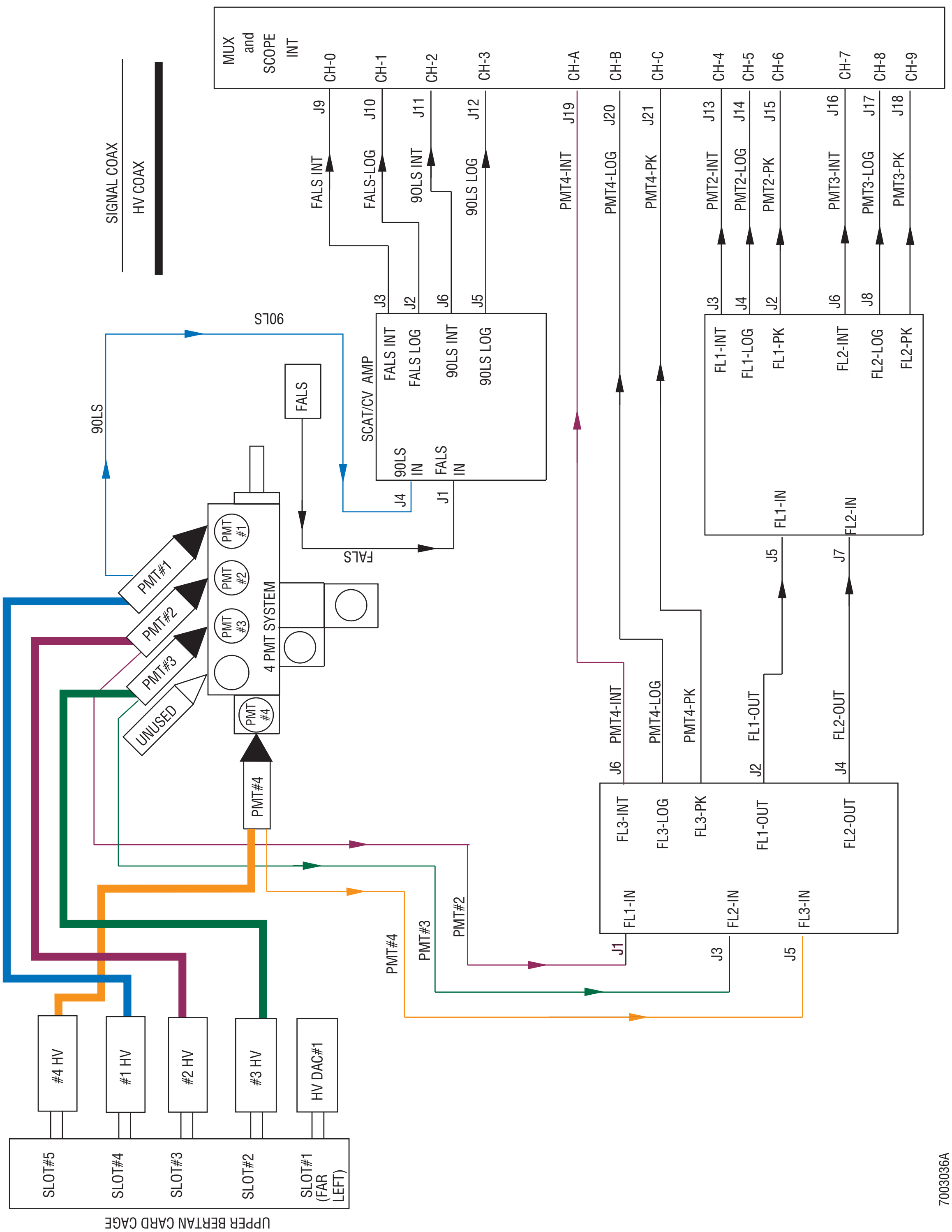
4 PMTs, Nonswitchable Amps

The 4PMT, Nonswitchable Amp (Figure 2.6-8) is the earliest configuration of the Elite.

With this setup:

- All fluorescence PMT signals go to the 3 PMT Sub Amp card.
- Coax cables take FL1 and FL2 to the Dual FL Amp card for linear and log amplification.
- The 3 PMT Sub Amp card completes the FL3 signal processing.
- Coax cables route all processed signals to the Mux and Scope card.
- The Scat/CV Amp card processes all FALS and 90LS signals.

Figure 2.6-8 4PMT, Non-Switchable Amps without Peak Scatter



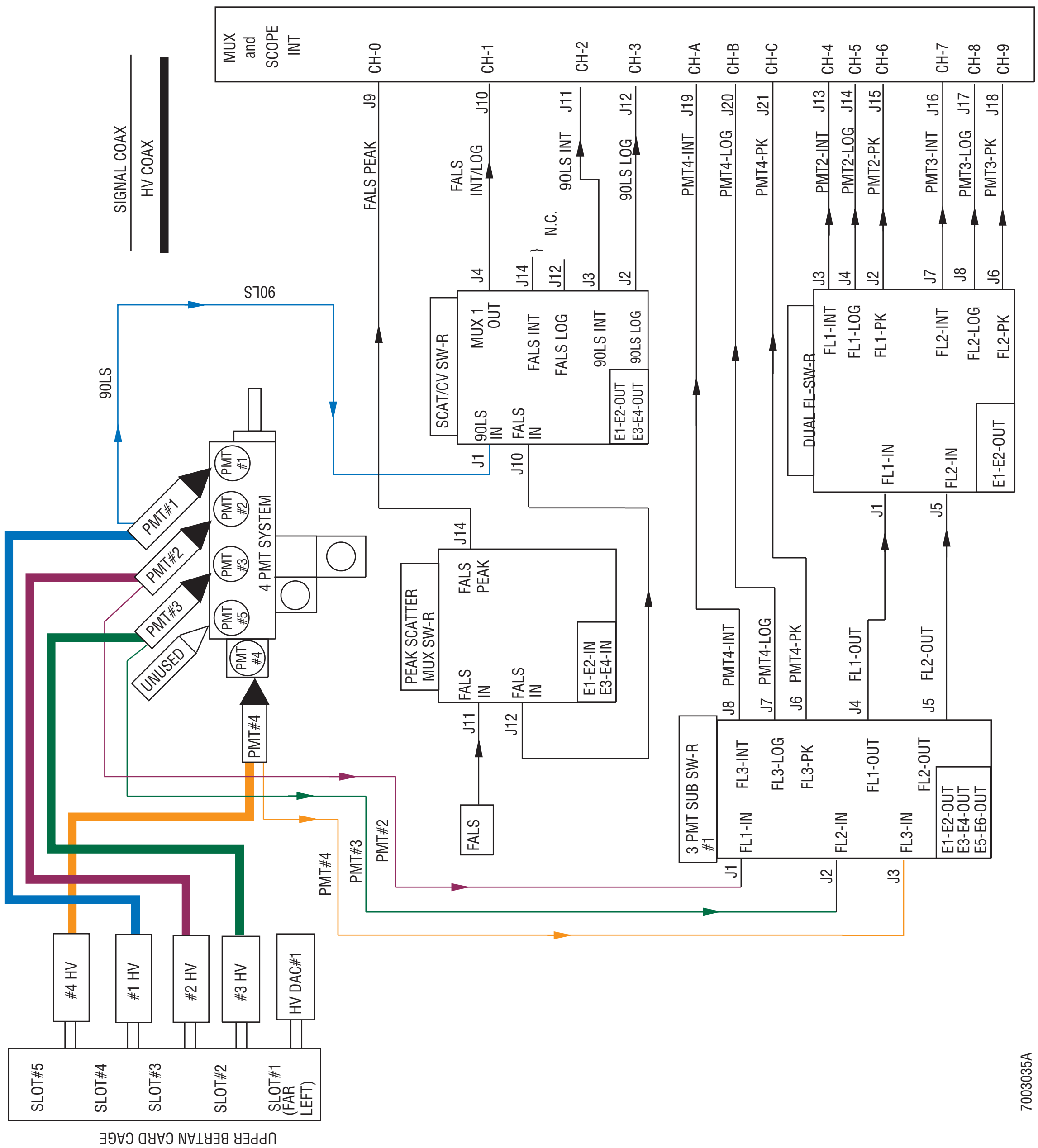
4PMTs, Switchable Amps with Peak Scatter Function

The 4PMT, Switchable Amps with Peak Scatter Function (Figure 2.6-9) was a factory configuration available beginning February 1992.

With this setup:

- All fluorescence PMT signals go to the 3 PMT Sub SW-R card, which compensates all three signals, FL1, FL2, and FL3.
- FL1 and FL2 are routed to the Dual FL SW-R card for linear and log amplification.
- The 3 PMT Sub Amp card completes processing the FL3 signal.
- Coax cables take all processed signals to the Mux and Scope card.
- This configuration uses the peak scatter FALS sensor.
- Coax cables carry the FALS signal to the Peak Scatter/Mux SW-R card.
- The Peak Scatter/Mux SW-R card splits the signal in two:
    - One signal is routed to J12 and then to the Scat/CV SW-R card, which processes the signal to provide the integral and log FALS signals.
    - The second signal leaves the Peak Scatter/Mux SW-R card from J14, where coax cables carry the signal directly to the Mux and Scope card.

Figure 2.6-9 4PMT, Switchable Amps with Peak Scatter



5PMTs, Nonswitchable Amps, No Gated Amp

The 5PMT, Nonswitchable Amp, No Gated Amp (Figure 2.6-10) was the first field-upgradable configuration.

With this setup:

- The system must now subtract from four signals to provide compensation, which it does by routing the FL2 and FL3 signals to both PMT Sub SW-R cards. As a reminder, with the 4PMT, nonswitchable Amps setup there were only three signals.
- A second 3 PMT Sub SW-R card processes the additional PMT.
- The 3 PMT Sub SW-R 1 card processes all combinations of FL1, FL2, and FL3.
- The 3 PMT Sub SW-R 2 card processes all combinations of FL2, FL3, and FL4.
- The system cannot compensate for FL1 and FL4 together because they are not routed to a common card. This is not a problem, however, since the two PMTs normally used optical filters with passbands far enough apart to prevent any spectral overlap.

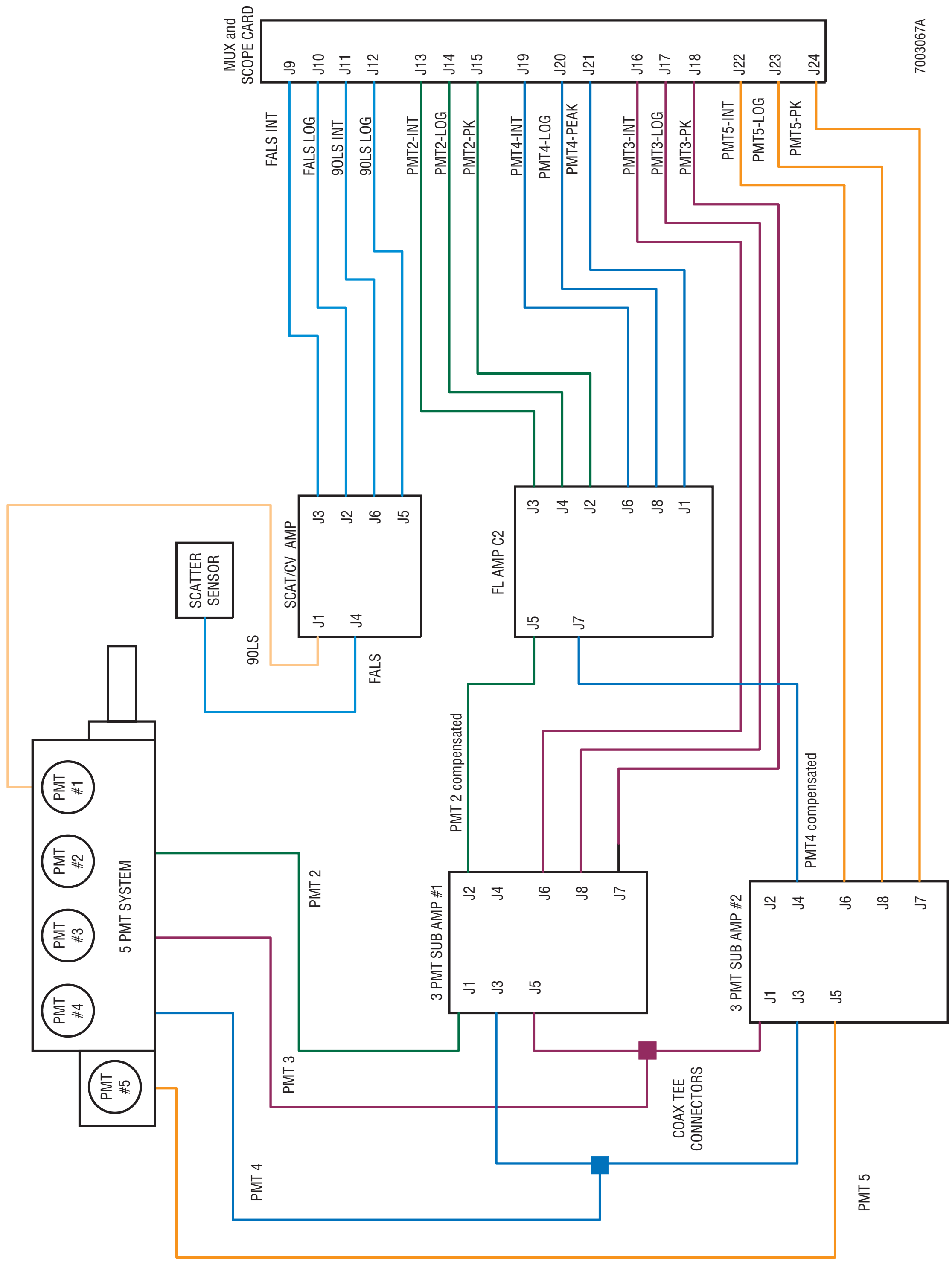


Figure 2.6-10 5PMT, Non-Switchable Amps without Peak Scatter

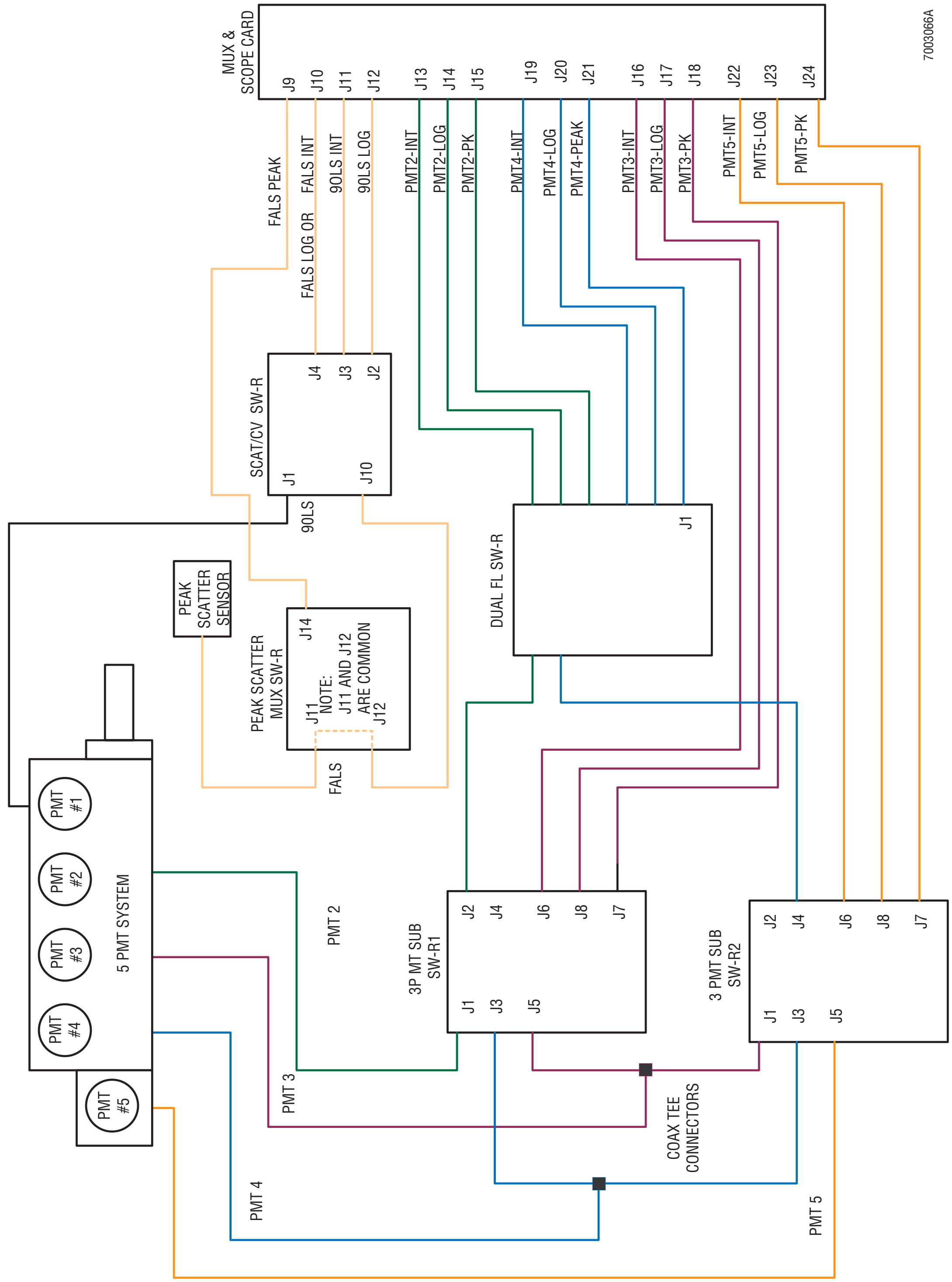
5PMTs, Switchable Amps, No Gated Amp

The 5PMT Switchable Amps (Figure 2.6-11) adds a fifth PMT and PMT Sub SW-R card.

With this setup:

- FL1 and FL2 are routed to the Dual FL SW-R card for linear and log amplification.
- The 3 PMT Sub SW-R card completes processing the FL3 signal.
- Coax cables take all processed signals to the Mux and Scope card.
- This configuration uses the Peak Scatter FALS sensor.
- Coax cables carry the FALS signal to the Peak Scatter/Mux SW-R card.
- The Peak Scatter/Mux SW-R card splits the signal in two:
  - One signal is routed to J12 and then to the Scat/CV SW-R card, which processes the signal to provide the integral and log FALS signals.
  - The second signal leaves the Peak Scatter/Mux SW-R card from J14, where coax cables carry the signal directly to the Mux and Scope card.

Figure 2.6-11 5PMT, Switchable Amps with Peak Scatter

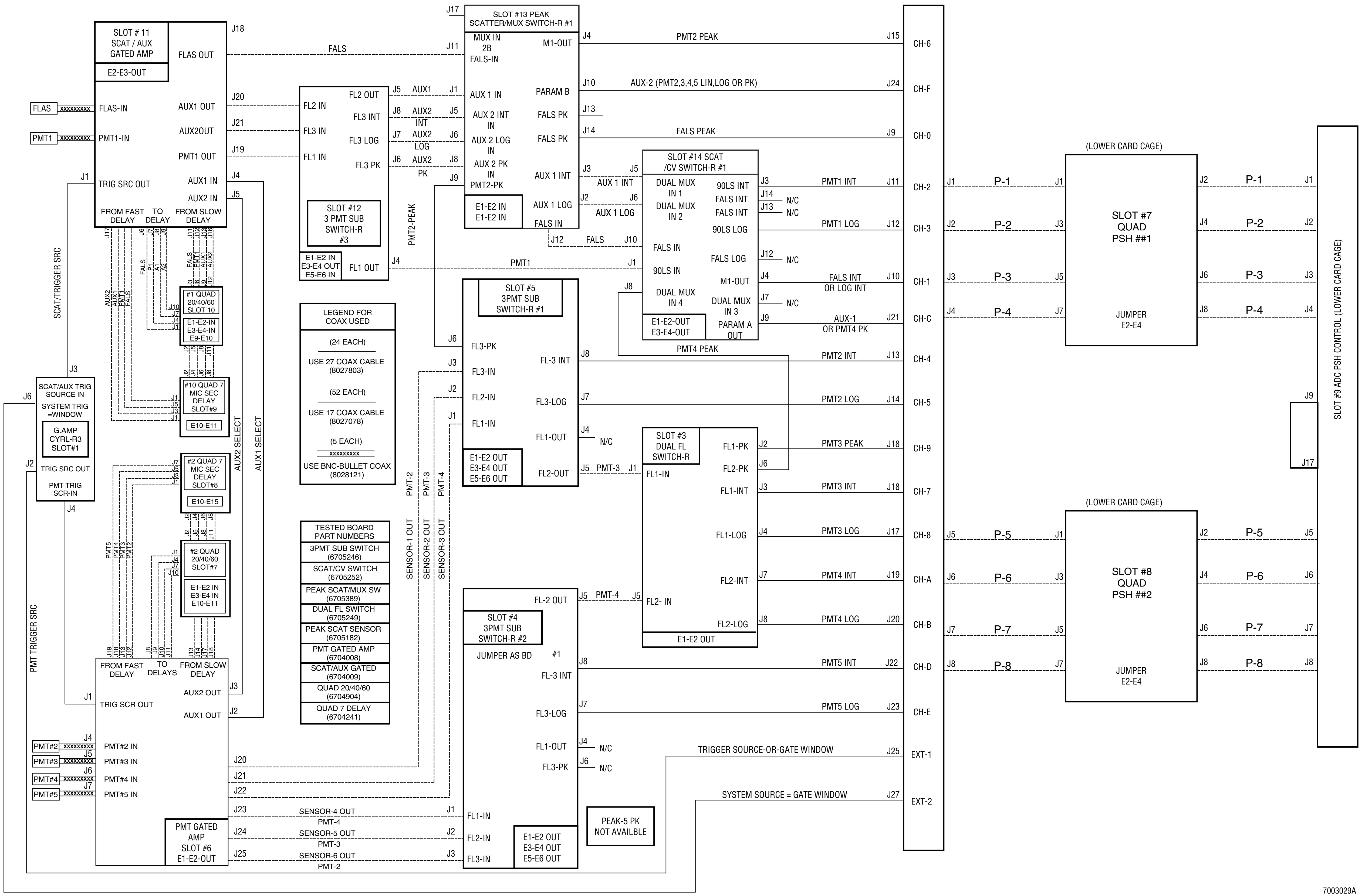


Gated Amp

Figure 2.6-12 is a block diagram that shows the circuit card signal interconnections for the Gated Amp system. The functions that each card performs appear in the individual card descriptions, including an explanation of how signals are selected and processed within the cards.

The illustration traces the FALS and PMT signals from where they enter on the left to where they pass into the Mux and Scope card on the right.

Figure 2.6-12 Block Diagram, Gated Amplifier



2.6-20

2.7 OPTICAL ELEMENTS

Overview

This section describes optical principles as they relate to the optical components used in the Elite system. The different aspects of the behavior of light are simplified to provide you with a basic understanding with minimum mathematical complexity.

Light

Definition

Light is a form of electromagnetic radiation with wavelengths that range from about 200 to 4,000 nanometers (nm). Wavelengths from about 400 to 700 nm are visible by human eye. Wavelengths shorter than 400 nm are ultraviolet (UV). Wavelengths longer than 700 nm are infrared (IR).

A unit (photon) of light is produced when an atom changes energy levels. In most light sources, many atoms change energy levels randomly.

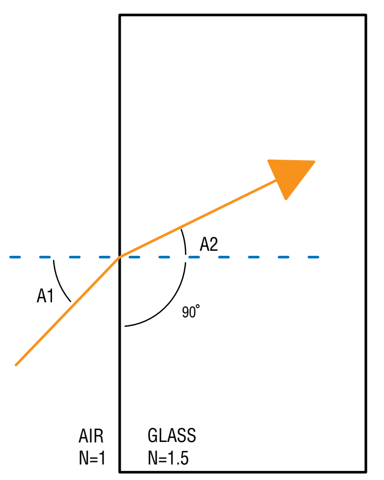
In modern optics, light consists of a dual nature. In some cases, light behaves as a wave, and in other cases, it behaves as a massless energy-carrying particle, a photon.

Media Used for Travel

Light travels through various media, such as air, glass, and water. Each medium has a property known as index of refraction n, where n is the ratio of the speed of light in air compared to that in the specific medium. As light travels obliquely from one media into another, the change in n changes the direction of the light; this is known as refraction. The principle of refraction makes it possible to trace the path of light rays and to understand the action of lenses. Refer to Lenses in this section for more information.

Figure 2.7-1 shows the refraction of a light ray as it passes from air into glass. Notice that the ray is bent toward a line perpendicular to the glass surface.

Figure 2.7-1 Refraction of Light



Snell's Law

Refraction at the interface between two optical media with indices of refraction n1 and n2 appears as the following according to Snell's Law:

$$n1 \sin \theta 1 = n2 \sin \theta 2$$

where the angle is measured normal to the surface.

In most materials, n is a function of the light's wavelength. This property is known as dispersion, and it allows a prism to separate colors. However, this is a problem for lenses, since lights of different colors refract differently and, therefore, focuses differently.

Lenses

The Elite uses two types of lenses: positive and negative.

Positive Lens

A positive lens has two convex spherical surfaces and:

- Reduces a laser beam to cellular size (since a laser is a source of parallel rays)
- Forms an image

Figure 2.7-2 shows the positive lens focus.

All parallel rays of light that enter the lens refract so they all converge on a point known as the focal point.

The lens works as well in reverse. The lens refracts those rays emitted from the focal point in varying directions into a parallel beam of light. The Elite's collection lens applies this principle to gather the fluorescent light leaving the cell into a parallel beam directed toward the filters and the PMTs.

Figure 2.7-2 Positive Lens Focus

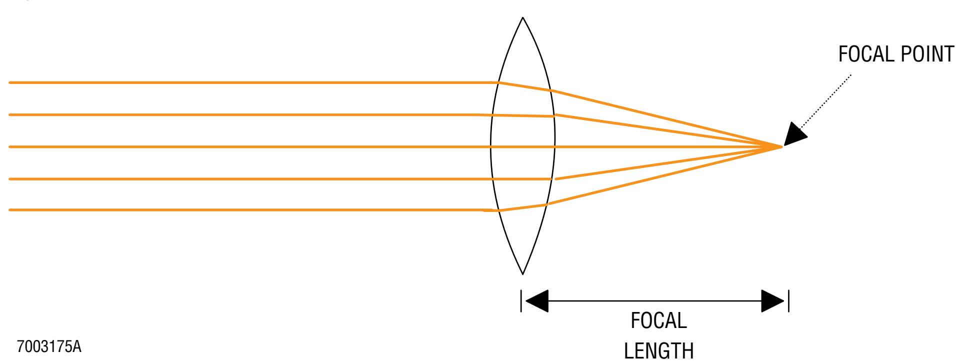
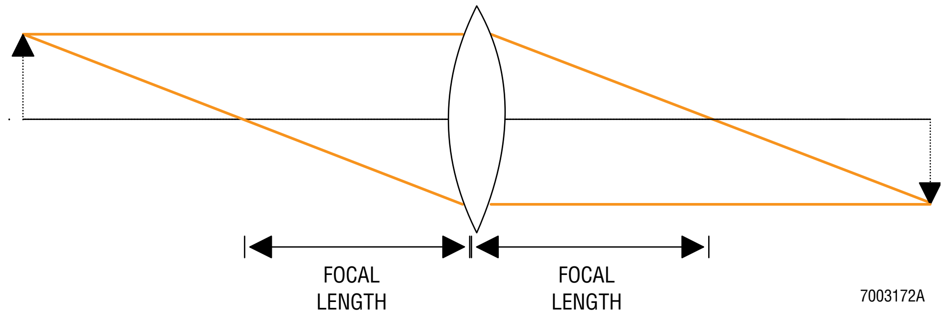


Image Formation: The camera lens projects and magnifies the sort stream onto the camera. Many rays of light leave a point on an object. However, for clarity in describing image formation, this example uses only two rays. Figure 2.7-3 shows how the positive lens forms an image.

Figure 2.7-3 Image Information



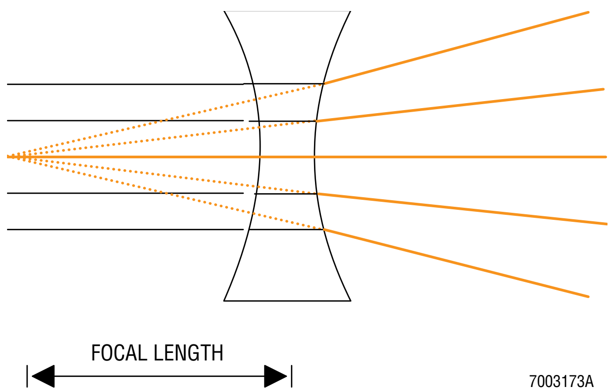
In the example, two light rays that leave a point on the object are traced through the lens. The top ray is parallel to the optic axis, and the lens refracts the ray to pass through the focal point. The bottom ray leaves the same point on the object and passes through the front focal point of the lens. Because this ray enters the lens from the focal point, the lens refracts the ray to be parallel with the optic axis. The lens forms one point of the image at the intersection of the top and bottom rays.

Note: All rays that leave the same point on the object and pass through the lens converge on the same image point.

Negative Lens

A negative lens (Figure 2.7-4) is a concave spherical surface and is opposite from the positive lens. A negative lens refracts rays of light so they diverge or spread out. The point from which these rays appear to diverge is the focal point.

Figure 2.7-4 Negative Lens Focus



Equations

There are two equations that apply to the lenses used in the Elite: the Lensmaker's equation and the Thin Lens equation.

The Lensmaker's equation is:

$$\frac{1}{f} = (n - 1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$$

where,

f = focal length
n = index of refraction (1 for air; 1.5 for glass)
R_1 = radius of the positive lens
R_2 = radius of the negative lens

The more curvature a lens has (smaller R), the smaller the focal length (f).

The Thin Lens equation is:

$$\frac{1}{f} = \frac{1}{p} + \frac{1}{q}$$

where,

f = focal length
p = distance of an object
q = image

The Thin Lens equation relates the focal length to the distance of an object (p) and distance from the lens where an image (q) forms. Magnification is determined by -q/p.

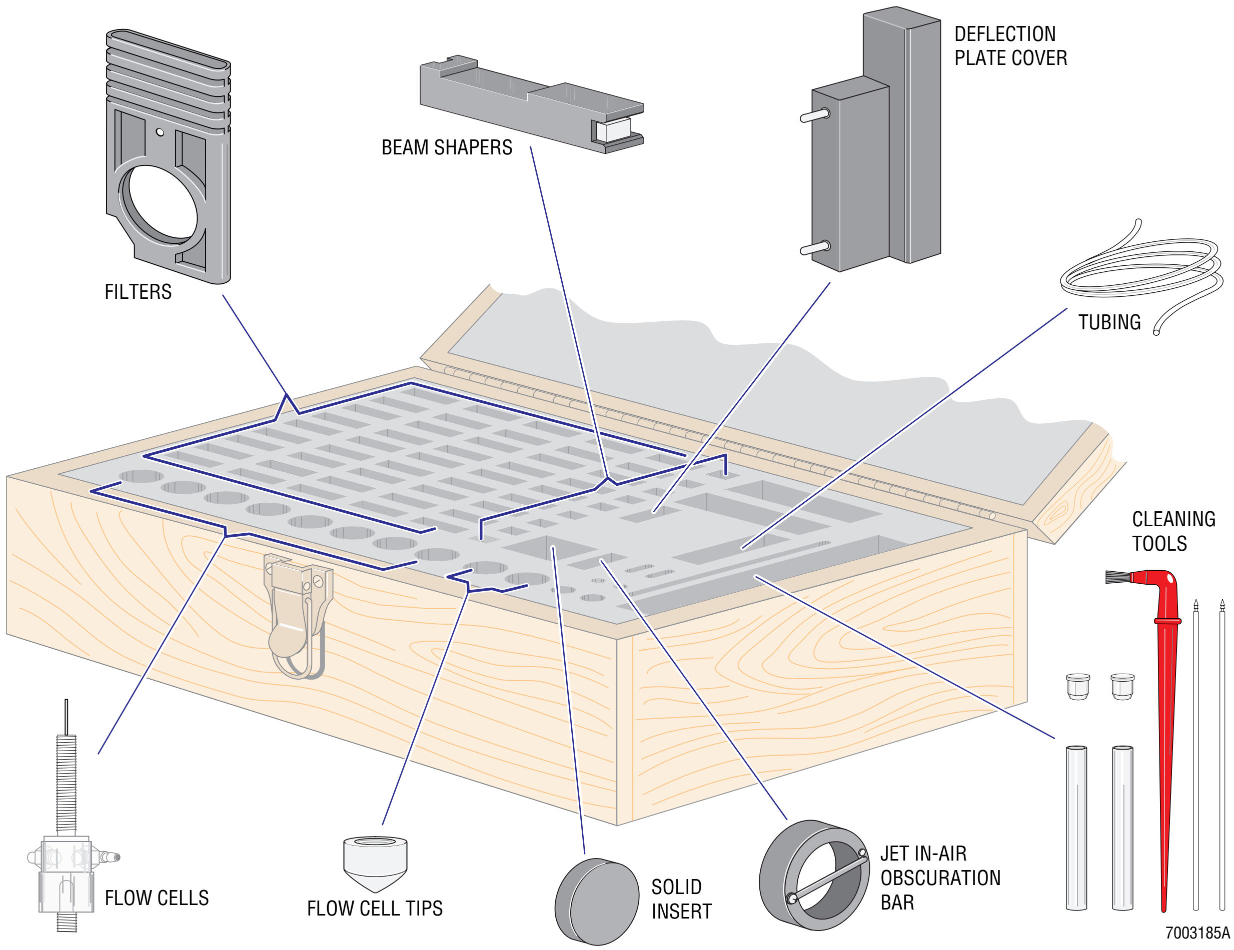
Optical Components Used in the Elite

The Elite uses the following optical elements:

- Beam shapers
- Beam expanders/reducers
- Parallel plates
- Lasers
- Flow cell tips

Figure 2.7-5 illustrates some of the optical accessories a customer may receive, depending upon the configuration of their instrument.

Figure 2.7-5 Optical Accessories



Beam Shapers

According to the refraction theory, parallel rays focus to an infinitely small point at the focal point. However, light is a form of radiation with a finite “size”, its wavelength. According to the wave propagation theory, the “spot” size that a laser beam can be focused to is:

$$K = \frac{\lambda f}{D}$$

where,

K = constant related to lens properties
λ = wavelength of laser
f = focal length of lens
D = diameter of laser beam.

Beam shapers use a combination of two cylindrical lenses that are made with one flat surface and one cylindrical surface; there is no spherical surface on a beam shaper. Since the glass curves in only one direction, refraction occurs in only one direction. By using two oppositely oriented lenses, it is possible to tailor the horizontal and vertical spot sizes. Figure 2.7-6 shows the beam shaper’s optics.

Figure 2.7-6 Beam Shaping Optics

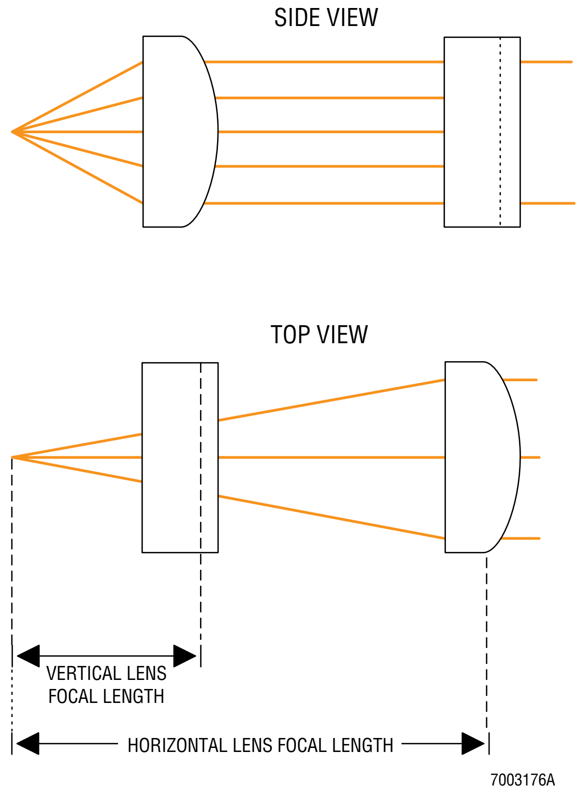


Table 2.7-1 shows some typical applications for different beam shapers.

Table 2.7-1 Typical Applications of Beam Shapers

Sorting Application	Flow Cell	Laser Power	Beam Shaper Spot Size	DISCSAT EXT.	Band Width	PPU Sensitivity	Data Rate
Lymphocytes with high intensity staining	76 µm jet-in-air or fast quartz	200 mW water-cooled	15 x 33 µm	2.5 µmsec	High	0.5 - 4.0 µmsec	High
Lymphocytes with low intensity staining	76 µm sort sense	15 mW air-cooled	15 x 60 µm	10 µmsec	Low	10- 20 µmsec	Low
Tissue culture/hybridoma cells/20 - 40 µm diameter	140 µm sort sense	15 mW air-cooled	15 x 80 µm	15 µmsec	Low	>20 µmsec	Medium
Enrichment (high-speed sorts)	76 µm Fast Quartz	200 mW water-cooled	15 x 33 µm	2.5 µmsec	High	0.5 - 4.0 µmsec	High
DNA stained with PI	100 µm Sort Sense	15 mW air-cooled	8 x 80 µm	5 µmsec	Low	4.0 - 10.0 µmsec	Low

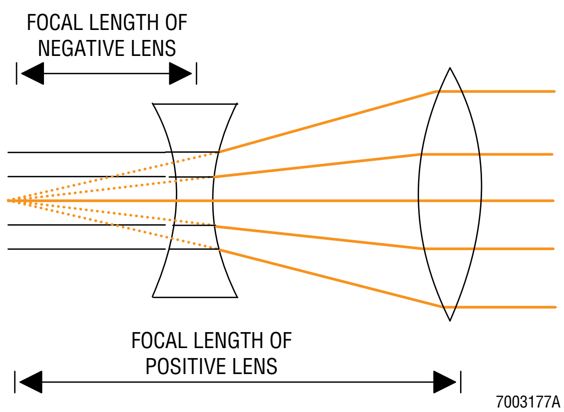
Table 2.7-1 Typical Applications of Beam Shapers *(Continued)*

Sorting Application	Flow Cell	Laser Power	Beam Shaper Spot Size	DISCSAT EXT.	Band Width	PPU Sensitivity	Data Rate
Chromosomes	76 or 100 µm Sort Sense	50 mw - 457 nm 50 mW - UV	6 x 100 µm	5 µmsec	Low	4 - 10 µmsec	Low
Stem cells Bone marrow	100 µm Sort Sense	15 mW air-cooled	15 x 80 µm	5 µmsec	Low	4 - 10 µmsec	High

Beam Expander/Reducer

This optical system of the beam expander/reducer (Figure 2.7-7) consists of a positive and negative lens. The lenses are situated so that their focal points overlap.

Figure 2.7-7 Beam Expander/Reducer



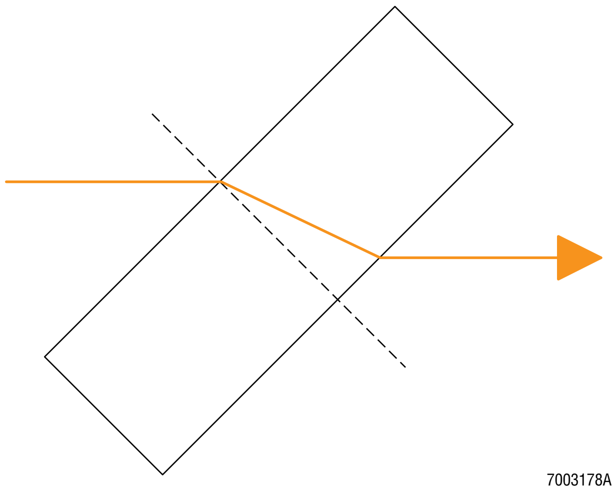
As a laser beam, which consists of parallel rays, enters the negative lens, it is refracted to diverge from the focal point of the negative lens. This results in a parallel laser beam with an increased diameter.

A beam entering from the other direction is reduced in diameter. The focal length ratio of the two lenses controls the amount of expansion/reduction. A beam expander/reducer has a focus adjustment that compensates for wavelength and allows you to set the desired amount of beam divergence.

Parallel Plate

Consisting of a thick piece of glass, a parallel plate translates a beam of light without altering the beam's direction. When a laser beam enters the plate at an angle, refraction bends the beam in a direction normal to the surface. The beam is refracted again by the same amount when exiting the glass (Figure 2.7-8).

Figure 2.7-8 Laser Beam Refraction



Light Sources

Light is produced when an atom changes energy level and emits a photon of light. The Elite uses a laser as a light source. A laser is a useful light source because it selectively amplifies light radiation in a narrow frequency and phase range. Similar to an array of antennas driven in phase by a single oscillator, laser light energy is in phase and is directional.

High Sensitivity Flow Cell Tips

As a sample passes through the laser spot, fluorescent light radiates outward in all directions. The mirror's radius is the same as the distance between the mirror's surface and the center of the flow channel. Therefore, emitted light striking the mirror reflects back toward the intersection point and toward the pickup lens.

The flow cell lens' focal length is greater than the distance between it and the intersection point. Used this way, the lens acts as a magnifier for the pickup lens. Because the flow cell lens is close to the light source, it intercepts a greater percentage of the total radiated light and relays the light to the pickup lens.

2.8 SORT SUBSYSTEM

Function

The Sort subsystem (Figure 2.8-1):

- Evaluates each event against previously loaded sort conditions
- Generates the appropriate signals for droplet formation and droplet deflection.

Overview

Independent Operation

Before the sort operation begins, the Multibus CPU card sets up the cards for the Sort subsystem. During the actual sorting process, the Sort subsystem operates without CPU intervention in response to each event.

Sort Cycle

The sort cycle goes through the following processes:

- The cell passes through the laser beam.

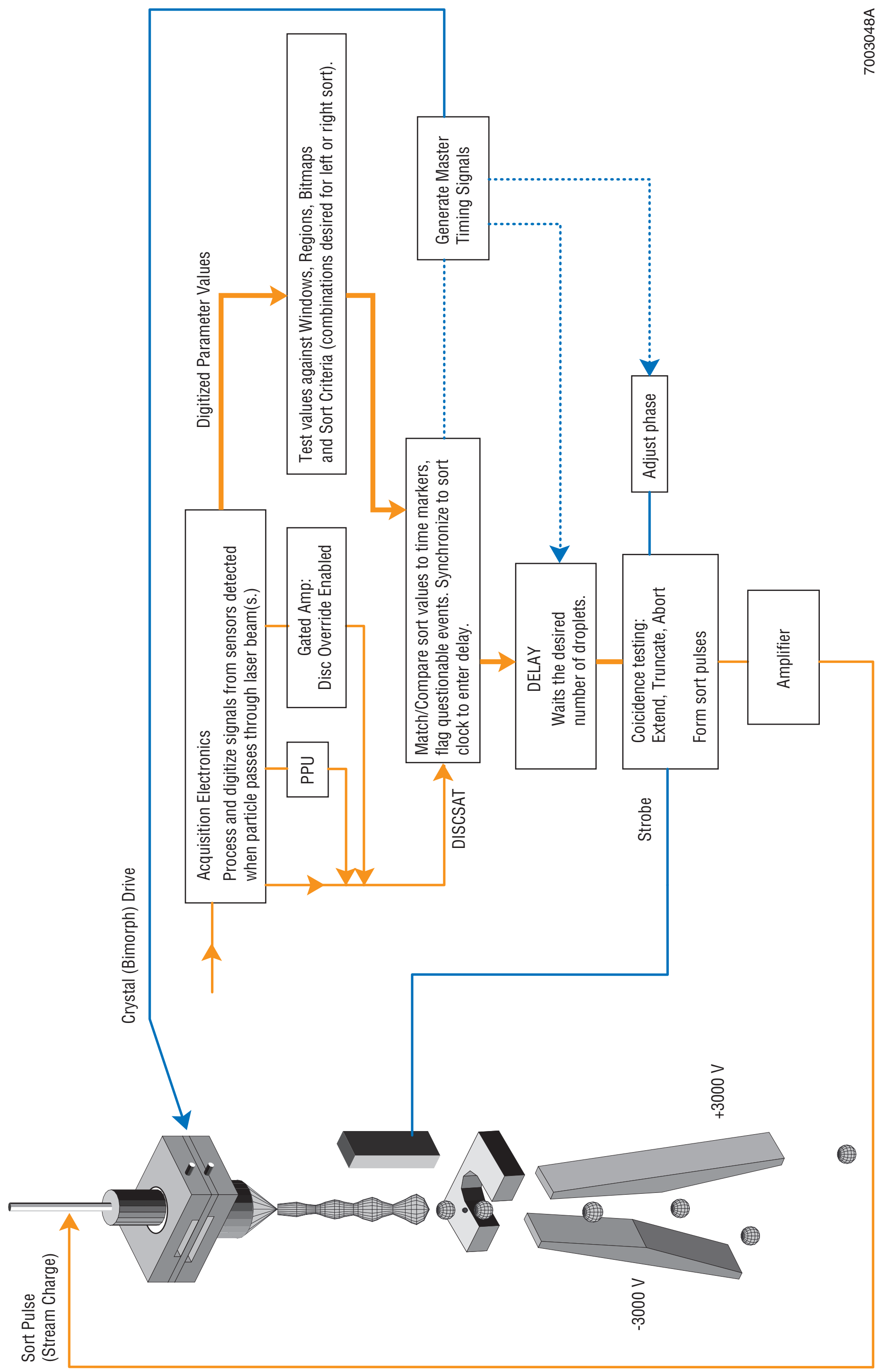
  The Acquisition subsystem indicates this time with the DISCSAT signal, usually originating in the Quad PSH card; it can also originate in the Gated Amp Control card and the Pulse Pileup Det./TOF card. The acquisition electronics measure selected parameters and place the digitized parameters on the data path. The sort electronics perform the following sequence for each cell:

  - Tests the parameter values for linear gates
  - Tests the parameter values for bit maps
  - Tests the logic conditions (sort criteria) and makes a final decision to sort
  - Waits for the cell to be in the last attached droplet
  - Tests the cell for coincidence
  - Deflects the droplet containing the desired cell.

Circuit Cards

Figure Figure 2.8-2 shows the Sort subsystem circuit card functions.

Figure 2.8-1 Sort Subsystem Overview



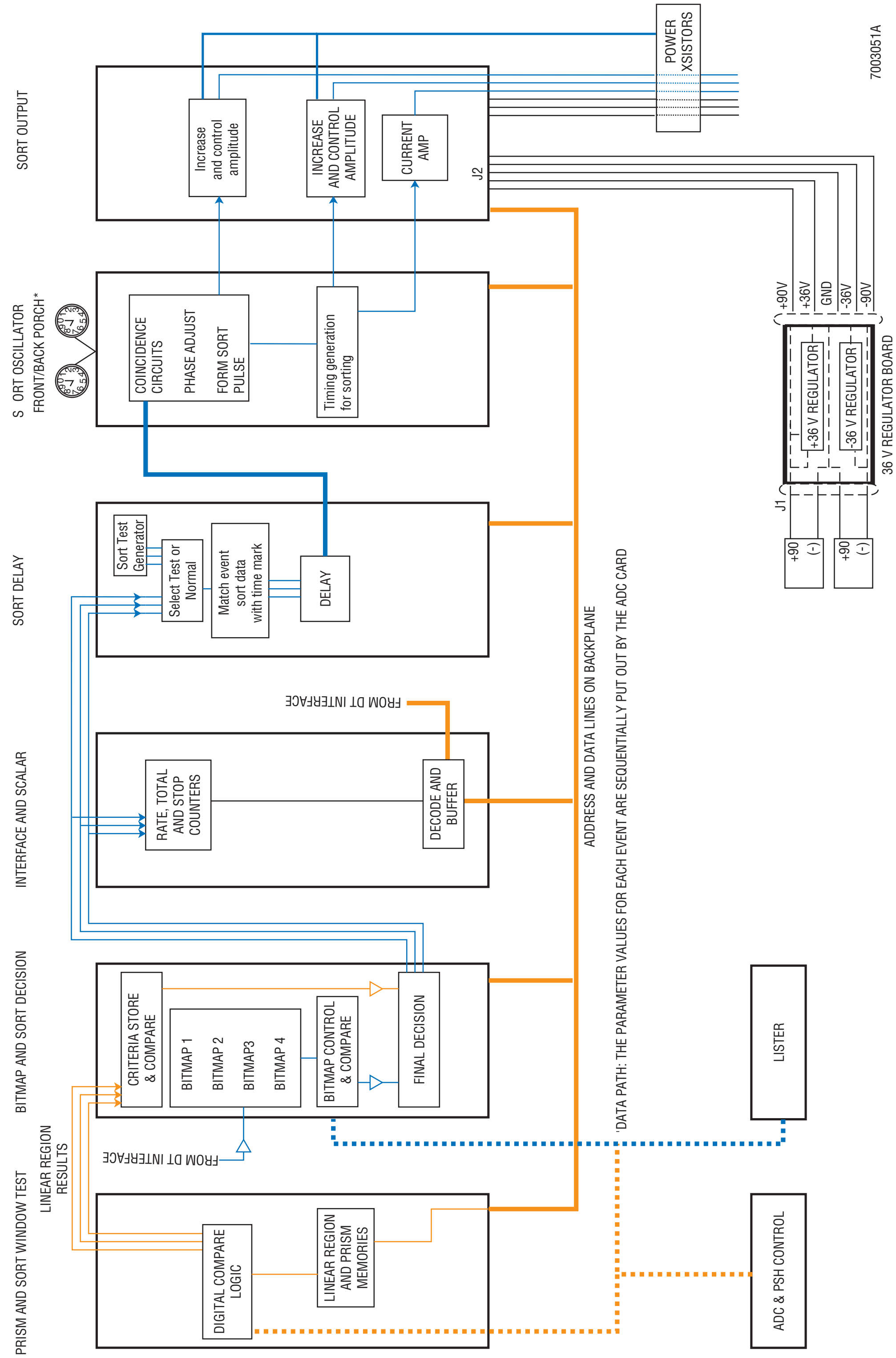


Figure 2.8-2 Block Diagram, Sort Subsystem Card Functions

The following text addresses the sorting process through the following circuit cards. For more detailed information, see the circuit schematics listed below:

Note: For the part numbers and location of these schematic files, see Chapter 6.

- Prism and Window Test card
- Bitmap and Sort Decision card
- Interface and Scaler card
- Sort Delay card
- Sort Oscillator card
- Sort Output card
- Pulse Pileup Det./TOF card

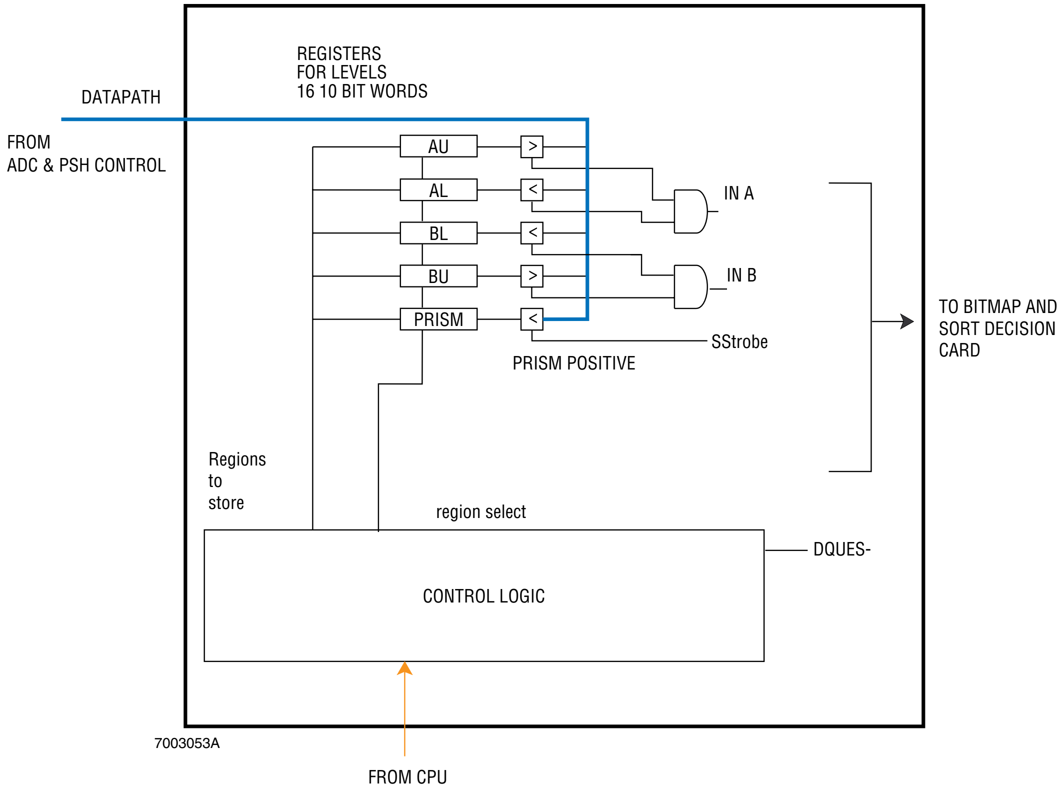
Prism and Sort Window Test Card

If rectilinear sort gates are used, the sort process begins at the Prism and Sort Window Test card (Figure 2.8-3), which:

- Stores the channel numbers that define the upper and lower limits for the sort regions being used. The storage occurs before the sort begins, when the Sort Settings are sent from the Workstation.
- Compares the values that the ADC presents on the datapath bus to the appropriate stored value (matching parameter) during the actual sort. The sort condition for a particular window and particular parameter is met when:

  current value > low channel number
  current value < high channel number.
- Sends the indication to the Bitmap and Sort Decision card if the sort condition is met. Current channel values are compared to the prism level for each parameter via a similar process.

Figure 2.8-3 Block Diagram, Prism and Sort Window Test Card



In Figure 2.8-3:

- The digital words representing the channel number for each parameter are put on the Datapath bus one at a time.
- The registers for levels provides storage for 8 sort regions.
- Digital comparators compare the current parameter values to the stored regions.
- The S-strobe indicates the comparison is complete.
- The DQUES are regions changed during sort.
- The Control Logic circuit, before sorting, stores sort regions sent from the CPU in the registers. During sort, the appropriate regions are compared to the current parameter values.

Bitmap and Sort Decision Card

Before a sort begins, the system loads the Bitmap and Sort Decision card (Figure 2.8-4) with the sort logic criteria, which is the information the Workstation sends to the Cytometer. The information contains the combination of gates and/or bitmaps that must be met so that a cell is sorted to the right or to the left.

This card also contains the memory where the four available sort bitmaps are stored. The system loads these memories before the sort begins. The user draws an amorphous region on

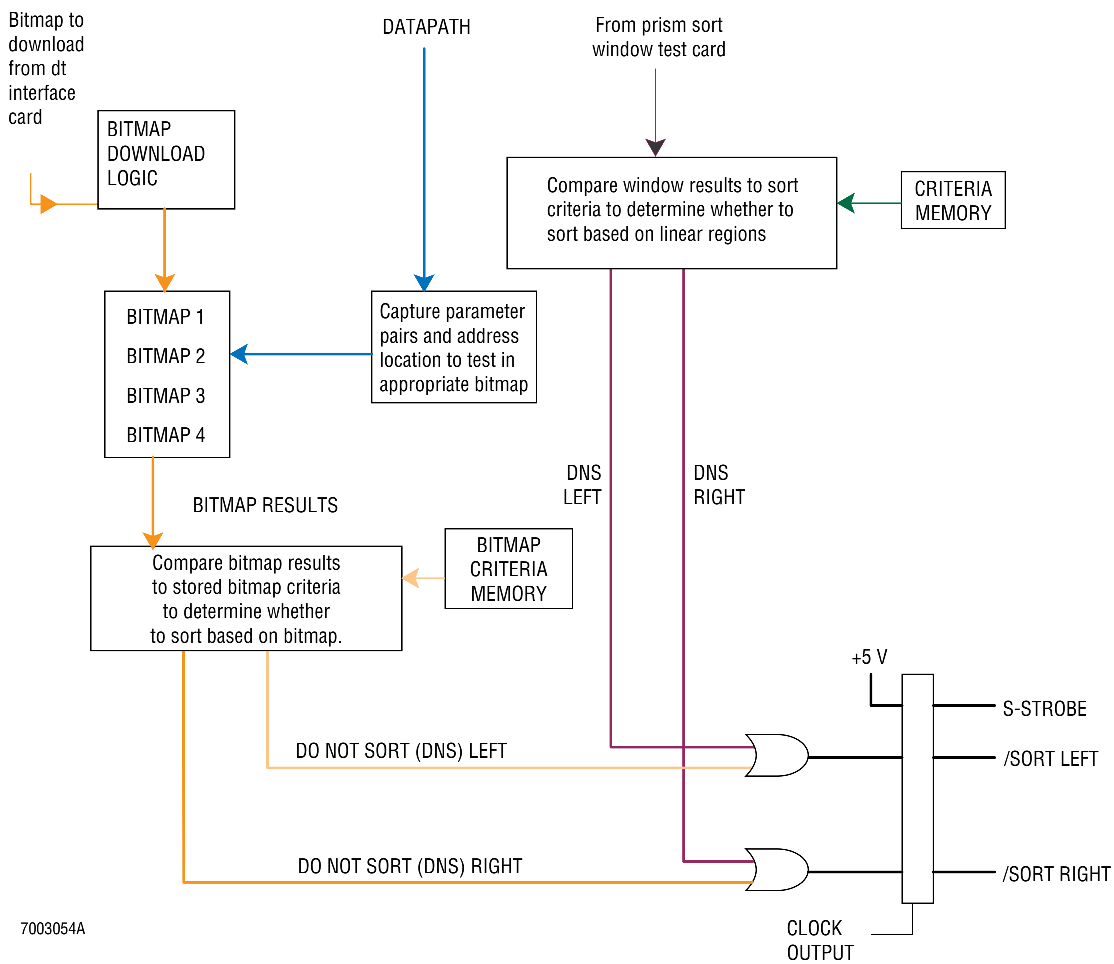
a two-parameter histogram background. Each X- and Y- channel number pair comprises a bit map location. The Elite software assigns a bit map location a 1 value if it is within the region and a 0 value if it is out of the region.

The Workstation then transfers the single-bit representation (bitmap) to the Cytometer. The Cytometer sends the pattern via the DT Interface card to one of the bit map memories. During the actual sort, the system tests each cell. Essentially, the card uses the channel numbers for the two appropriate parameters as coordinates to access the stored bit map and to read the stored bit. If the system reads the bit as a 1, then the event has fallen within the amorphous region. If the card does not read the bit as a 1, then the event has not fallen within the amorphous region. A considerable amount of logic circuitry is needed:

- To latch in the correct parameter channel numbers as they appear on the dataPath bus
- To use these values as memory addresses into the proper bitmap memory
- To latch out the appropriate results.

The card then compares the results to the stored sort criteria in order to reach a second partial decision to sort based on bitmaps. The decision to sort based on bitmap comparisons and sort logic are combined with the linear region results to reach a final sort decision, which is, does the cell meet all gate and region conditions set for a right or left sort. The card sends this decision to the Sort Delay card for synchronization to system droplet generation.

Figure 2.8-4 Block Diagram, Bitmap and Sort Decision Card



In Figure 2.8-4, the bitmap download logic decodes the incoming data word to address and set a particular location in a bitmap.

Interface and Scaler Card

The Interface and Scaler card:

- Provides digital counters for various sort actions: sort right, sort left, abort counts, %right, and %left.
- Compares the sort counts to the sort stop values. When the card reaches the stop value, it signals the Sort Oscillator card to stop the sort.
- Records other system events via counters, including acquisition elapsed time and acquisition stop count.

Sort Delay Card

The Sort Delay card (Figure 2.8-5):

- Delays the application of the sort pulse until the cell to be sorted is in the correct place.

  This function is complicated because cells appear in the laser beam at random times, while a droplet can only be sorted at the time it breaks off from the end of the stream. Therefore, the randomly appearing sort requests must be delayed and synchronized with the droplet formation rate.

- Receives input from the Bitmap and Sort Decision card indicating an event should be sorted right or left.

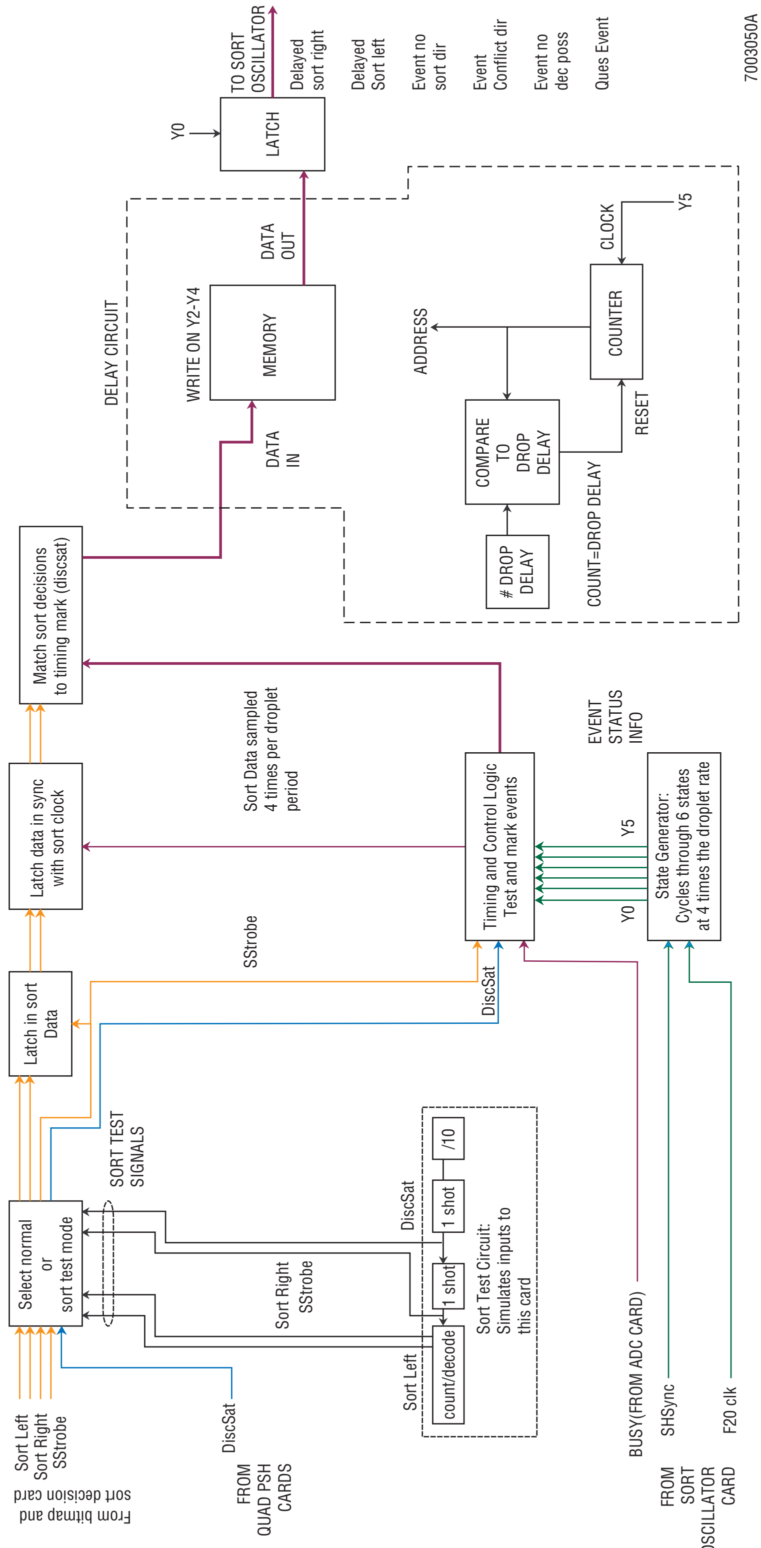
  The card synchronizes the inputs to the digital clock signals received from the Sort Oscillator card. These clock signals are synchronous with the droplet formation rate but at a higher frequency (the f20 clock).

- Delays the synchronized sort signals in a type of shift register for the user-selected number of droplet periods.

  The key is that the card uses a clock frequency that is synchronous with the droplet formation rate. The card samples the sort signals and loads them into the delay memory at four times the formation rate. This provides the system with a quarter drop sort resolution.

- Sends the delayed sort signals to the Sort Oscillator card.

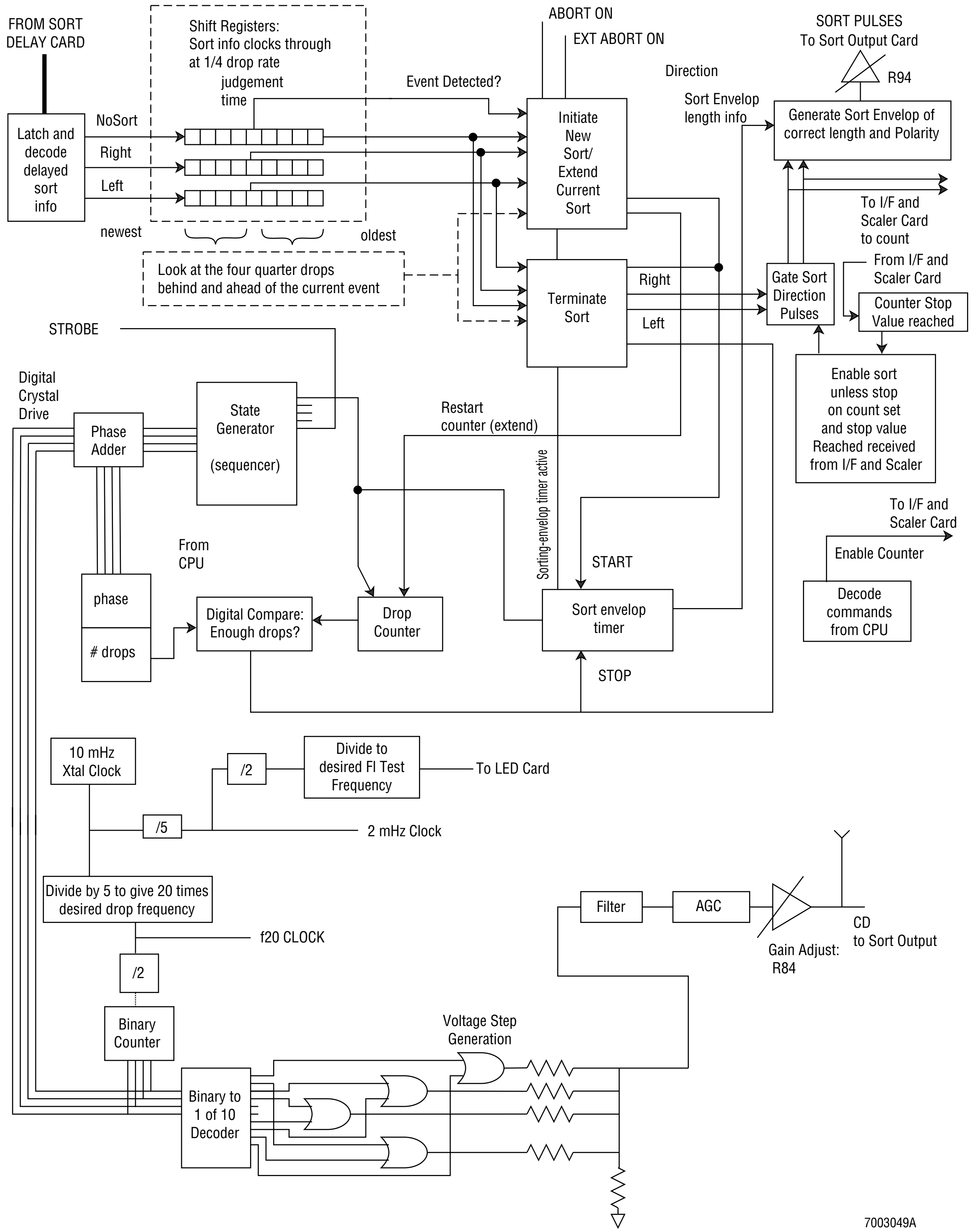
Figure 2.8-5 Block Diagram, Sort Delay Card



Sort Oscillator Card

Coincidence Logic: The delayed sort information from the Sort Delay card comes into the Sort Oscillator card (Figure 2.8-6) and is clocked into the card at quarter droplet intervals.

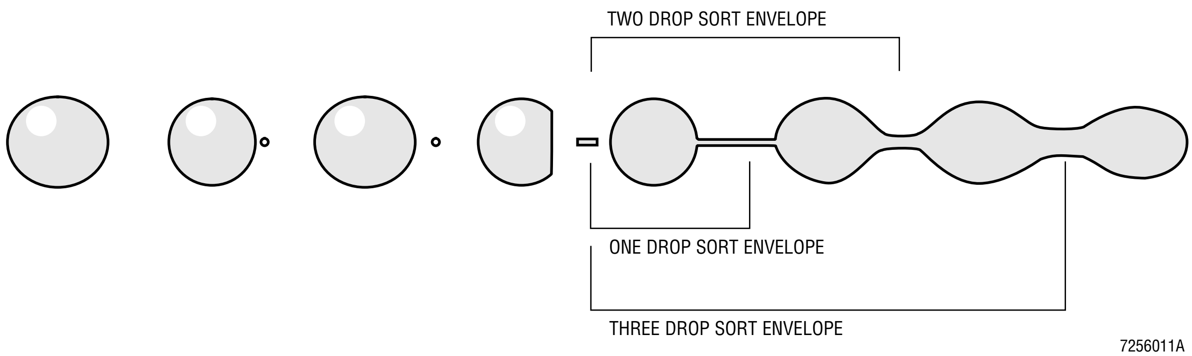
Figure 2.8-6 Block Diagram, Sort Oscillator Card



The logic circuits examine the sort event information and the controls settings to either generate:

- A sort window envelope (Figure 2.8-7) of the user preprogrammed length: 1, 2, or 3 drops
- No expected sort envelope because an abort condition has occurred (abort),
- A sort window envelope longer than preprogrammed (sort extension), or
- A sort window envelope shorter than preprogrammed (truncation).

Figure 2.8-7 .Sort Envelope



Function

The Sort Oscillator card:

- Generates various timing signals for sorting.

  A crystal oscillator provides a stable time base. A variable digital divider (rate multiplier) divides the clock to generate the droplet breakoff clock frequency. The clock times sorting using circuits on this card and on the sort Delay card.

  The digital clock also creates a synthesized sine wave signal that drives the bimorph. This signal goes to the Sort Output card for amplification. The strobe drive signal is also derived from this clock.

  A second programmed divider generates the FL test frequency.
- Processes the delayed sort signals received from the Sort Delay card.

  The Sort Oscillator card processes the delayed sort signal received from the Sort Delay card to provide the fine delay adjust (phase). When enabled, the abort circuitry looks one drop period (four quarters) ahead and one drop period (four quarters) behind the quarters containing the desired sort signal to detect possible coincident events. The card aborts all non-identical coincident events.

  To accomplish this task, a short shift register allows the local circuitry to simultaneously examine nine quarter droplets:

  - One quarter droplet where the event in question lies
  - Four quarter droplets ahead
  - Four quarter droplets behind

Note: For Elite ESP systems, abort can be set to eliminate all potentially coincident events. The Elite ESP surrounds all detected events with an abort window, this includes cells that would and would not be sorted.

- Transforms the sort pulse from TTL-type signals to an analog bipolar signal. The front and back porch signals are generated in this process.

  Note: The user's choice of Number of Drops controls the length of the sort pulse. This sort signal then goes to the Sort Output card for amplification.

Three coincident conditions can occur. See Table 2.8-1.

Table 2.8-1 Coincident Condition Responses

Condition*	Response	System Response	Explanation
Good cell followed by a good cell This means that both cells meet the criteria to sort in the same direction.	Extension	System attempts to sort both cells.	The logic circuit extends the sort pulse to sort the second cell if the second cell falls in another droplet adjacent to the first cell's sort window (more than four quarter droplets away. In a one-droplet sort, if the second cell falls in the next drop, the sort window extends to form a two-droplet sort. A three-droplet sort can extend to five droplets. **Note:** Extension occurs under the above conditions in any sorting coincidence mode, including Abort OFF. (Extension is actually an unabort.) **Note:** With Abort ON (in any mode), extension will not occur if the cells are close enough together to fall in the same droplet, which means within four quarter droplets from one another. In this case, the system aborts both cells.
Good cell followed by a bad cell This means that a cell does not meet all the criteria to sort in the same direction as the first cell.	Truncation	System sorts the good cell, but not the bad cell.	The system truncates the pulse if: The system is performing a sort of at least two droplets, and The bad cell occurs in one of the droplets in the first cell's sort window, and Abort is ON (any mode except Abort OFF).

**Definitions:*

Good = a particle or cell that meets the conditions to be sorted; meets the sort equation by being in the correct regions and/or bitmaps.

Bad = a particle or cell that does not meet the conditions to be sorted.

Direction = left or right.

Droplet = as a measure of time, the reciprocal of the droplet frequency gives the period of one droplet. At 32 KHz, one droplet period is 1/32 KHz; therefore, 1/32 KHz = 31 microseconds. This allows you to relate the time difference between pulses as observed on the scope relative to how far apart they will be in the sort stream in droplets.

Followed-by = implies the second cell is close enough to the first cell to create a sorting problem.

Table 2.8-1 Coincident Condition Responses *(Continued)*

Condition*	Response	System Response	Explanation
Bad cell followed by a good cell This means that the bad cell does not meet the criteria to sort in either direction.	Abort with Complete Abort **not** ON		If Abort is ON and Complete Abort is not selected an abort occurs only if the bad cell is in the same droplet as the good cell, which means no more than four quarter droplets away. **Note:** It is possible for a bad cell to occur early enough in the first droplet and not be detected by the system, which means the system sorts it with the good cell (when greater than a 1-drop sort envelope is chosen).
	Abort with Complete Abort ON		If Abort is ON and Complete Abort is selected, an abort occurs if the droplet window that the bad cell would have generated and the droplet window for the good cell overlap. For example: In a three-droplet sort, the cells abort if they are closer than four droplets apart. This permits a bad cell in the first droplet to be detected, resulting in the system aborting both cells.

**Definitions:*

Good = a particle or cell that meets the conditions to be sorted; meets the sort equation by being in the correct regions and/or bitmaps.

Bad = a particle or cell that does not meet the conditions to be sorted.

Direction = left or right.

Droplet = as a measure of time, the reciprocal of the droplet frequency gives the period of one droplet. At 32 KHz, one droplet period is 1/32 KHz; therefore, 1/32 KHz = 31 microseconds. This allows you to relate the time difference between pulses as observed on the scope relative to how far apart they will be in the sort stream in droplets.

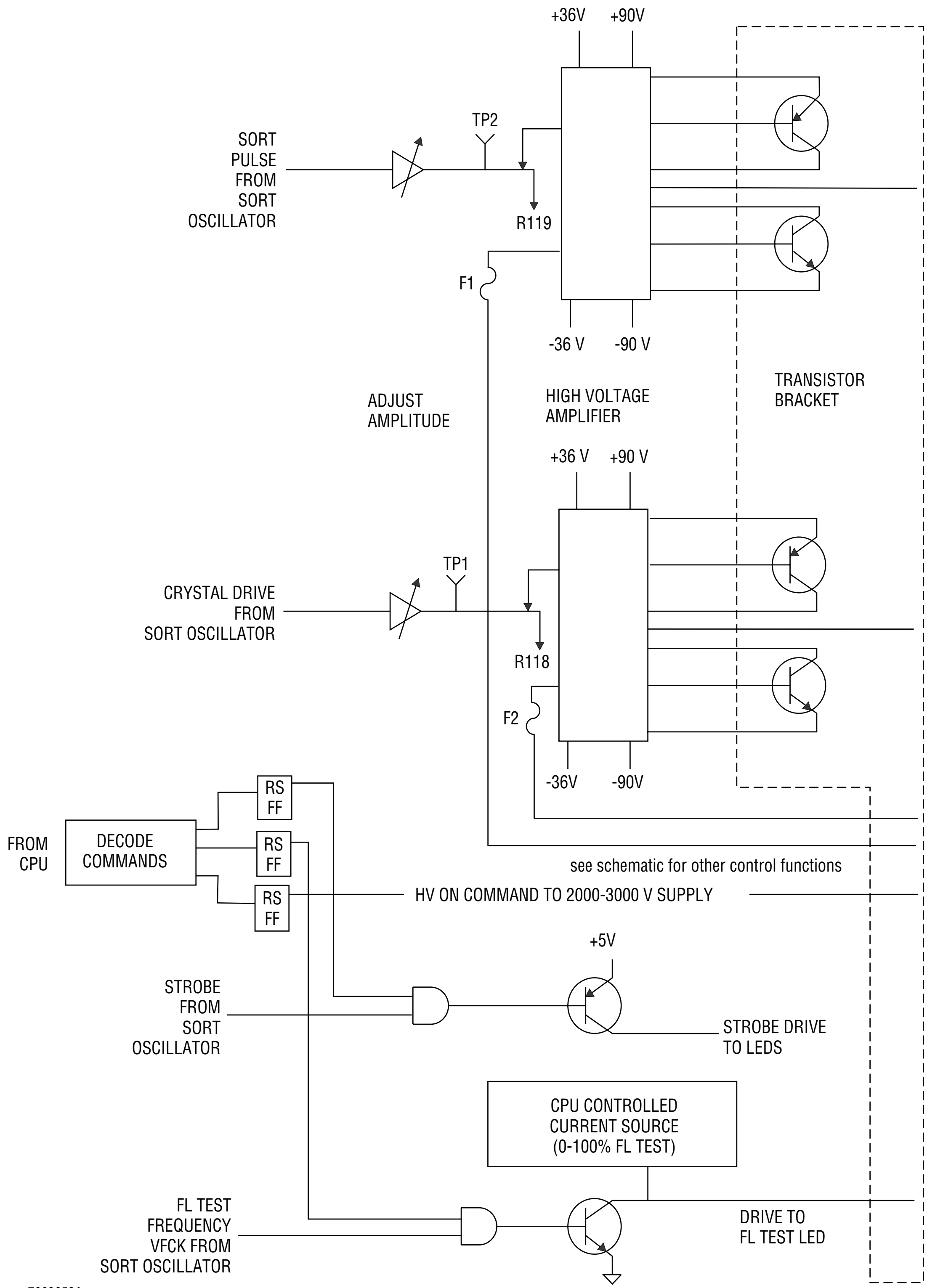
Followed-by = implies the second cell is close enough to the first cell to create a sorting problem.

Sort Output Card

The Sort Output card (Figure 2.8-8):

- Amplifies the crystal drive and sort pulse signals to appropriate voltage levels
- Controls the amplitude of these signals to provide the crystal drive and sort deflection controls.
- Buffers the FL test and strobe signals from the Sort Oscillator card to provide outputs suitable to drive LEDs.
- Adjusts the FL test signal's amplitude based on operator input.

Figure 2.8-8 Block Diagram, Sort Output Card and Transistor Bracket Card



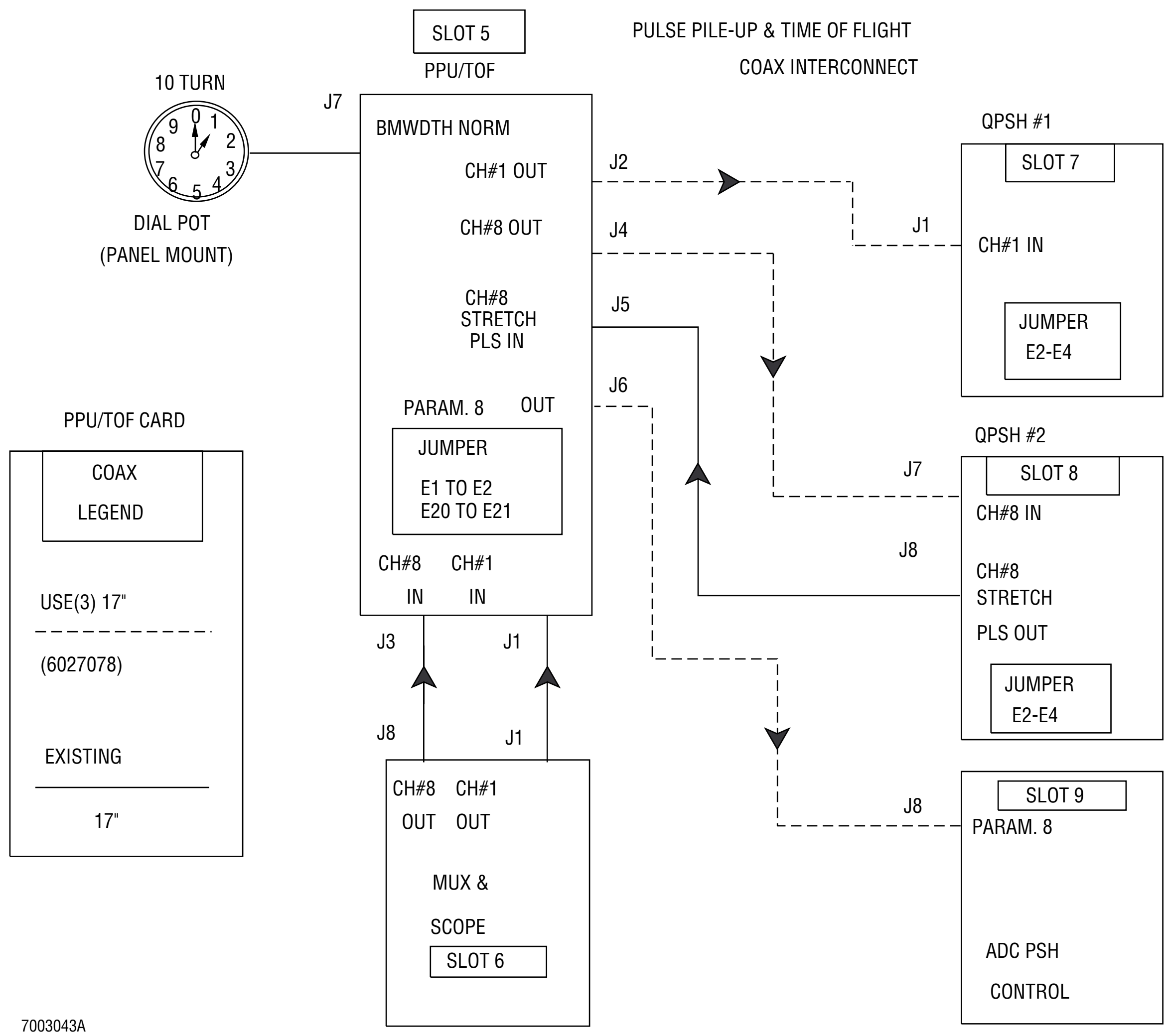
Pulse Pileup Det./TOF Card

The Pulse Pileup Det./TOF card card)(Figure 2.8-9) performs two major functions:

- TOF (time of flight) provides an output voltage proportional to the length (duration) of the input pulse. This function applies to particle sizing and doublet detection. See TOF Function for additional information.
- PPU detects pulses (cells or particles) that occur closer together than can be detected with current techniques. This function helps improve sort purity by aborting events where contamination could otherwise occur. See PPU Function for additional information.

  Note: When PPU is enabled, the PPU parameter (PAR#1) generates a positive PPU detect if that parameter is greater than 9.8 V (or channel 1,000).

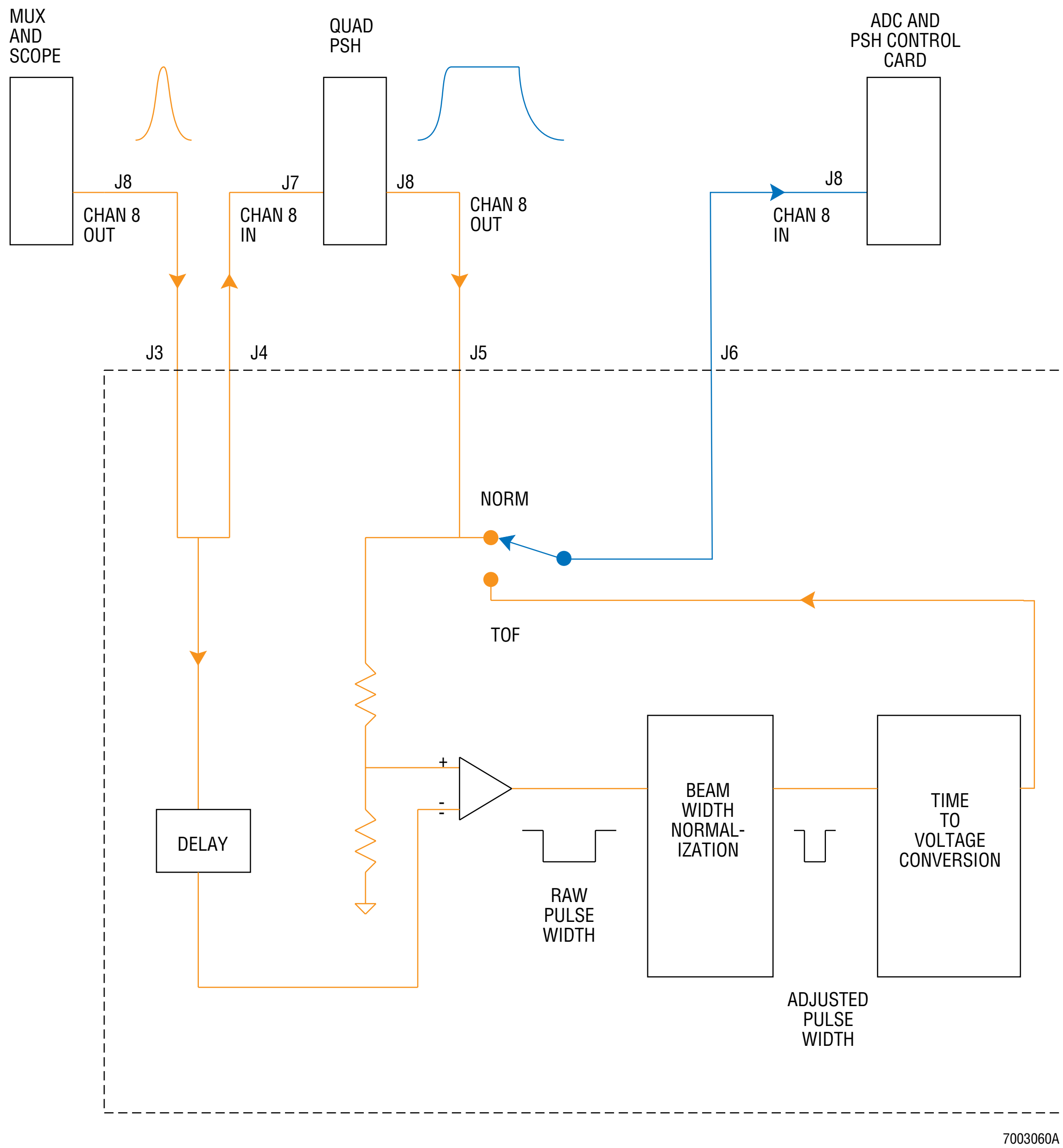
Figure 2.8-9 Interconnection of Pulse Pileup Det./TOF Card



TOF Function

The TOF function (Figure 2.8-10) of the Pulse Pileup Det./TOF card measures the length of the signal assigned to parameter eight.

Figure 2.8-10 TOF Function of Pulse Pileup Det./TOF Card



The output voltage of the card is proportional to the length of time that the pulse exceeds 14% of its own amplitude. The stretched pulse output from PSH8 goes to a resistor voltage divider to develop the 14% reference level that is applied to one input of a voltage comparator. The comparator initially sees the reference level on its positive input and outputs a high. As the delayed input pulse rises above the reference level, the comparator output goes low until the input pulse falls back below the reference level.

As the particle enters the laser beam, the signal pulse rises to its peak value; it does not drop until the particle leaves the laser beam. Since each particle in the sample flows through the laser at the same speed, the card actually measures the diameter of the particle by measuring how long it takes that particle to pass a certain point. However, since the laser beam has a finite diameter, the pulse length at the comparator output actually represents the time it takes the particle to pass through the beam. And since the beam diameter is constant (for a particular experiment), the system can subtract this time from each width pulse by the Beam Width Normalization circuit; this leaves a pulse that only represents the particle.

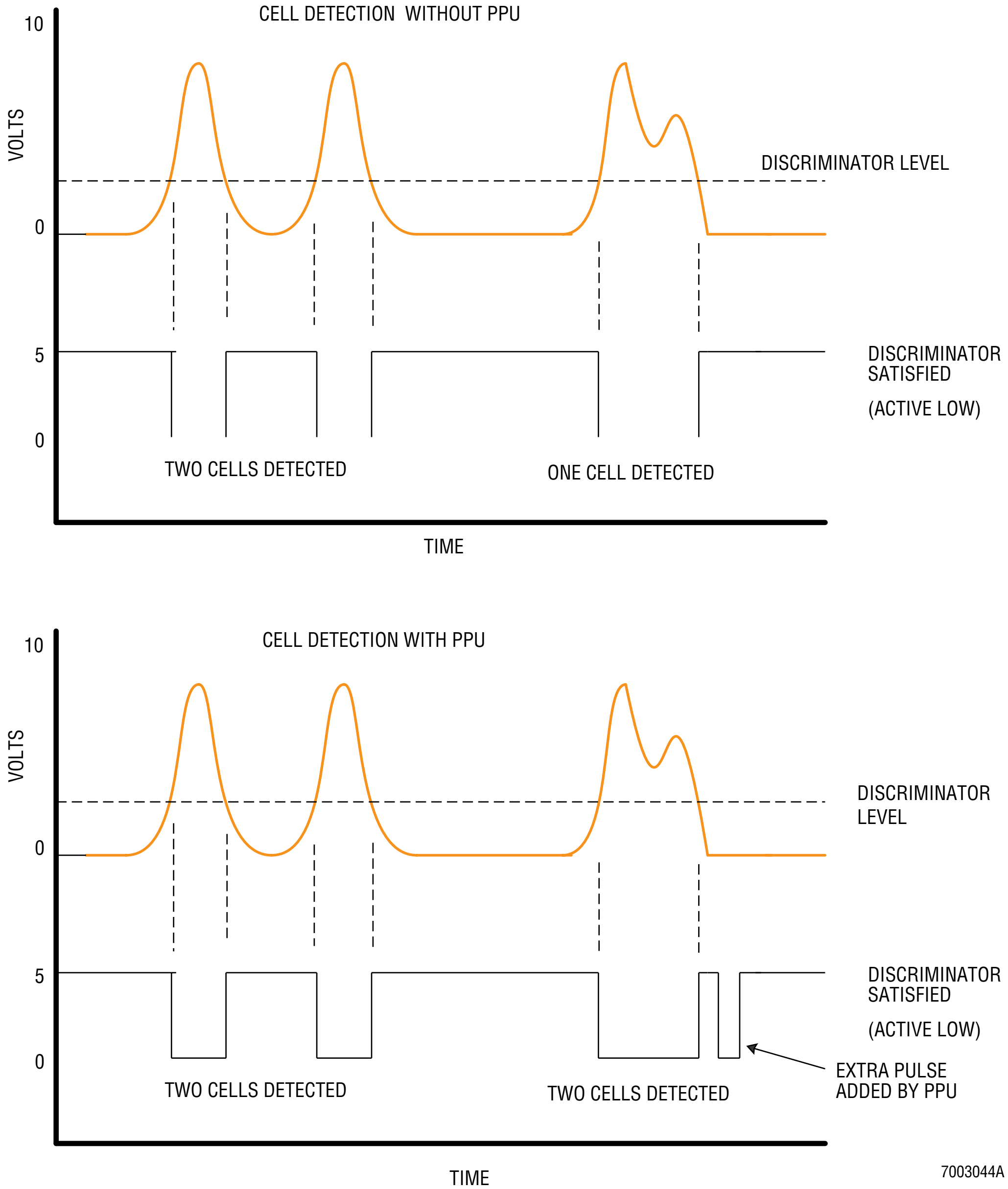
The card converts the pulse width to a voltage level that represents the length of the pulse. This allows the system to acquire the level as a parameter. The time-to-voltage circuit provides four scales to cover the range of possible pulse widths and particle sizes.

PPU Function

The PPU function (Figure 2.8-11) of the Pulse Pileup Det./TOF card monitors the pulse for changes in slope polarity. A single cell gives rise to one slope polarity change, which is at the peak when the slope changes from positive to negative. With a doublet, the slope changes polarity at the peak of the first cell, at the valley between the two cells, and at the peak of the second cell. When the PPU detects more than one slope change inside an event (as determined by discriminator crossings), the PPU generates an additional discriminator-satisfied signal for the event. The sorting electronics then use this information to abort a sort pulse, if Abort is ON.

Note: The PPU parameter also generates a positive detect output if the PPU parameter if that parameter is greater than 9.8 V (or channel 1,000).

Figure 2.8-11 PPU Function of Pulse Pileup Det./TOF Card



The system determines when it has seen a cell by monitoring discriminator crossings. A pulse in the parameter being used for the discriminator represents one even cell when it passes through the discriminator twice. One pass is on the rising edge, and the second pass is on the falling edge. Cells that are touching or that pass through the laser beam side by side give rise to a single long pulse that does not return to zero “between” cells. In this case, the pulse may not dip below the discriminator between cells, and the system does not recognize the second cell as a distinct cell.

Transistor Bracket Card

The final output power transistors for the crystal drive and sort pulse amplifiers are located on the Transistor Bracket card.

Note: All the connections from the card to the sort hardware are routed through this card.

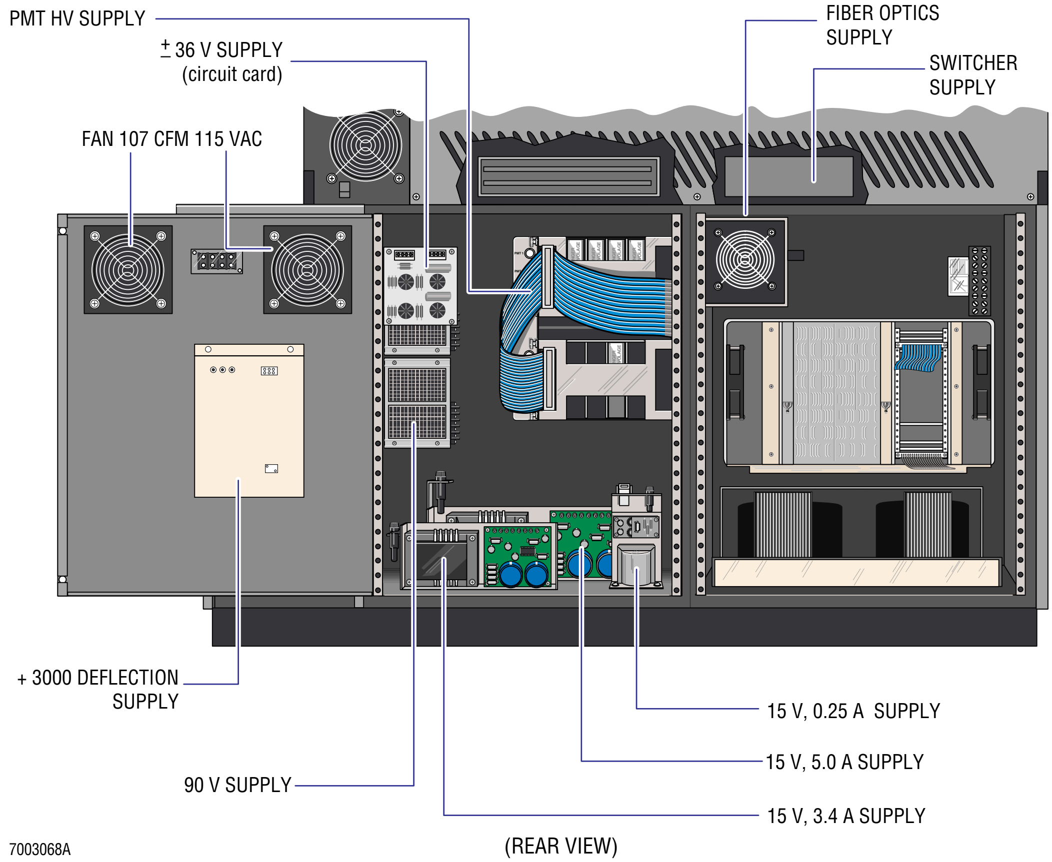
Power Supplies

Figure 2.8-12 shows the location of the power supplies, and Table 2.8-2 defines each power supply's function.

Table 2.8-2 Power Supply Functions

Power Supply	Function
±3000 V Deflection Power Supply	Charges the Deflection plates. Only active when turned on by Sort Output card.
±36 V Power Supply	Provides power to the Sort Oscillator card for bimorph oscillation and stream deflection.
90 V Power Supplies (2)	Two identical 90 V power supplies are used to power the Sort Output card. One supply provides positive output, and the other provides ±90 V grounded negative output to the card. The supplies also provide power to the 36 V Regulator card, which provides ±36 V to the Sort Output card.
15 V, 5 A Power Supply	Provides power to the card cage and to the motor controller for the camera.
15 V, 3.4 A Power Supply	Provides power to the DACs for the PMTs.
15 V, 0.25 A Power Supply	Provides power to the PMTs and the FS Detector.
5 V Power Supply	Provides power to the KIR3 card to make selections on the Control Screen and on the CPU.
12 V Power Supply	Provides power to the dual CRTs and to the CPU.
24 V Power Supply	Provides power to the solenoids.

Figure 2.8-12 Power Supplies, Location



2.9 PNEUMATICS SUBSYSTEM

General

The pneumatics system operates as follows:

Note: For more specific information, see the Pneumatic/Hydraulic Layout and Pneumatic/Hydraulic Layout Analyzer engineering schematics. For the part numbers and location of these schematic files, see Chapter 6.

- The Compressor module provides pressure and vacuum to the system. The compressor module has one electrical and three pneumatic connections. One connection is the air input for the compressor. The third connection is a vacuum return. The compressor output is connected to the cooling coil located under the tabletop.
- The cooling coil allows the heat that has built up in the compressed air to dissipate and also allows moisture in the compressed air to condense. The cooled air next passes through the air/water separator, where the condensed water is trapped and the dry air passes through.
- Valve 15, water drain, periodically removes the water trapped in the air/water separator.
- The system pressure switch monitors the air pressure after the air/water separator. This switch is adjustable and is nominally set to close when the pressure reaches 22 psi; it reopens when pressure drops to about 18 psi.
- The air goes to the manifold assembly where it supplies pressure to all the electrically controlled solenoid valves. For more information on valves, refer to Solenoid Valves.
- The system vents sample, sheath, and rinse tank pressures into the drip chamber. The drip chamber is vented to the atmosphere through a biofilter. If this filter clogs up, then pressure builds up in the drip chamber and the drip chamber switch detects the buildup. On the screen, this appears as *Drip Chamber Error.*
- The system draws air from the waste bottle through the waste filter. The water filter is a hydrophobic filter that clogs if the waste bottle overflows. This condition results in an increase in vacuum detected by the waste filter switch. On the screen, this appears as *Waste Filter Error.*

Solenoid Valves

Each solenoid valve performs a specific function. See Table 2.9-1 for a description of manifold valves 1 - 15. See Figure 2.9-1 for an illustration of valve operations.

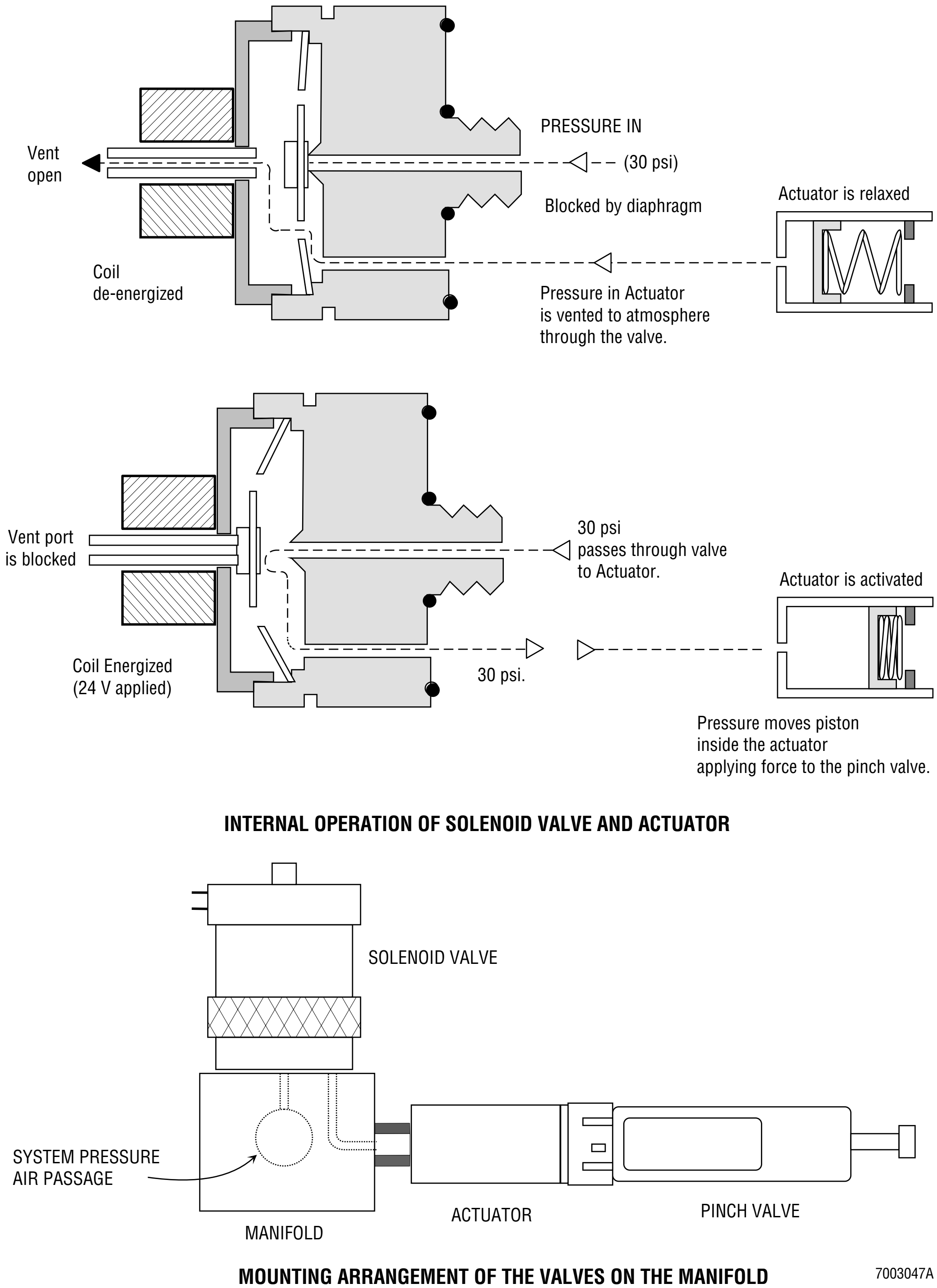
Table 2.9-1 Solenoid Valves, Manifold

Number	Name	Type	When Activated
VL1	System Pressure	Solenoid	Allows flow of air to the sheath and sample pressure regulators when energized.
VL2	Laser Shutter	Solenoid	Allows flow of air to laser shutter cylinder. Allows laser to pass when energized.
VL3	Sample Pinch	Solenoid	Provides air to the sample pressure pinch valve actuator, which activates the pinch valve to close the sample tubing.
VL4	Sample Pressure	Solenoid-operated double-acting pinch valve	Passes regulated sample pressure to the sample tube. When deactivated, it vents the pressure in the sample tube to the drip chamber.
VL5	Flow Cell Vacuum	Solenoid-operated single-acting pinch valve	Applies vacuum to the flow cell. Solenoid must be energized for pinch valve to close the vacuum tubing.
VL6	Sheath Pressure	Solenoid-operated double-acting pinch valve	Passes regulated sheath pressure to the sheath tank. When deactivated, it vents pressure in the sheath tank to the drip chamber.
VL7	Bubble Drain	Solenoid-operated single-acting pinch valve	Applies vacuum to the top of the bubble trap to remove any trapped air from the flow cell's path.
VL8	Rinse Pressure	Solenoid-operated double-acting pinch valve	Passes regulated sheath pressure to the rinse tank. When deactivated, it vents pressure in the rinse tank to the drip chamber.
VL9	Sheath Flow	Solenoid-operated single-acting pinch valve	Allows sheath to flow into the bubble trap because the pinch tube in the valve is released.
VL10	Sample Collect Drain	Solenoid-operated double-acting pinch valve	Allows waste to flow to the bottle when the tube connecting the drain to the waste bottled in unpinched.
VL11	Rinse Flow	Solenoid-operated single-acting pinch valve	Allows rinse liquid to flow into the bubble trap when the pinch tube in the valve is released.
VL12	Drip Chamber Drain	Solenoid-operated double-acting pinch valve	Applies vacuum to the drip chamber for draining when deactivated.
VL13	Autoclone Sorting Option Drain	Solenoid-operated double-acting pinch valve	Applies vacuum to either the Autoclone Sorting Option waste collector or to the sample station drain.
VL14	Autoclone Sorting Option Waste	Solenoid	Applies pressure to the Autoclone Sorting Option air cylinder, which pushes the waste collector forward.
VL15	Water Drain	Solenoid-operated single-acting pinch valve	Allows liquid in the air-water separator to drain into the waste bottle when deactivated.

**Unless otherwise noted, the valve performs when activated.*

Figure 2.9-1 illustrates valve operation.

Figure 2.9-1 Valve Operation



Circuit Cards

Pneumatic Interface Card

There are two communication pathways to the Pneumatic Interface card from the Multibus CPU card.

The first pathway is a direct cable connection between the Multibus CPU card and the Pneumatic Interface card. The Pneumatic Interface card buffers these signals into the appropriate 24 V drive levels to operate the solenoids.

The second pathway goes from the Acquisition card cage through the Sensor Interface card. This pathway allows the CPU to issue requested pressure to the Pneumatic Interface card and to receive information from the pressure sensors and switches.

The Pneumatic Interface card:

- Controls all pneumatic functions.
- Controls the solenoids. The card converts the logic level signals received from the CPU to 24 V levels that drive the fluidic solenoids.

  Note: A relay activated by the watchdog signals from the Sensor I/O card controls the 24 V.
- Controls the pressure: The card receives the requested sheath and sample pressure from the Multibus CPU card via the Sensor I/O card.

  After the values are received, the card achieves and maintains the requested pressures. Each system uses one of two types of pressure regulators and Pneumatic Interface cards. Refer to the chart below.

Card	Used With	Reference
Pneumatic Interface, PN 6704552	Norgren® pressure regulators	Old Type
Pneumatic Interface, PN 6705504	Low bleed regulators	New Type

See Heading 4.5, PNEUMATICS SYSTEM for additional information.

Sample Pressure Systems

There are two types of sample pressure systems: old type and new type.

Old Type

With the old type of sample pressure system (Figure 2.9-2), after you select the sample pressure, the CPU writes the information to the Pneumatic Interface card. The card retains this value and applies it to the digital-to-analog converter (DAC). You can read the DAC output with a DVM at test point 8 (TP8). To confirm operation of all the circuitry to TP8, you can monitor the test point while entering different samples pressure values through the Service -Valves screen.

If you cannot confirm operation of the circuitry, there is no need to replace any of the components beyond this point, including regulators. The exact voltage of TP8 is a function of the DAC reference voltage, which you can measure at TP1 and adjust via R34. Therefore, if TPI read -3.0 V, which is a typical value, and the requested sample pressure is 15 psi, then the

DAC output is 3.0 V. You can proportionally calculate the TP8 voltage for a pressure less than 15 psi: 10 psi yields 2.0 V, 5 psi yields 1.0 V, and so on.

The closed loop feedback system consists of the following:

- Sample regulator
- Sample pressure sensor
- Op amp network, known as the Driver circuit

Note: Only the Pneumatic Interface card circuitry totally controls the regulation process. Once the CPU and Sensor I/O cards provide a value to the Pneumatic Interface card, their roles end.

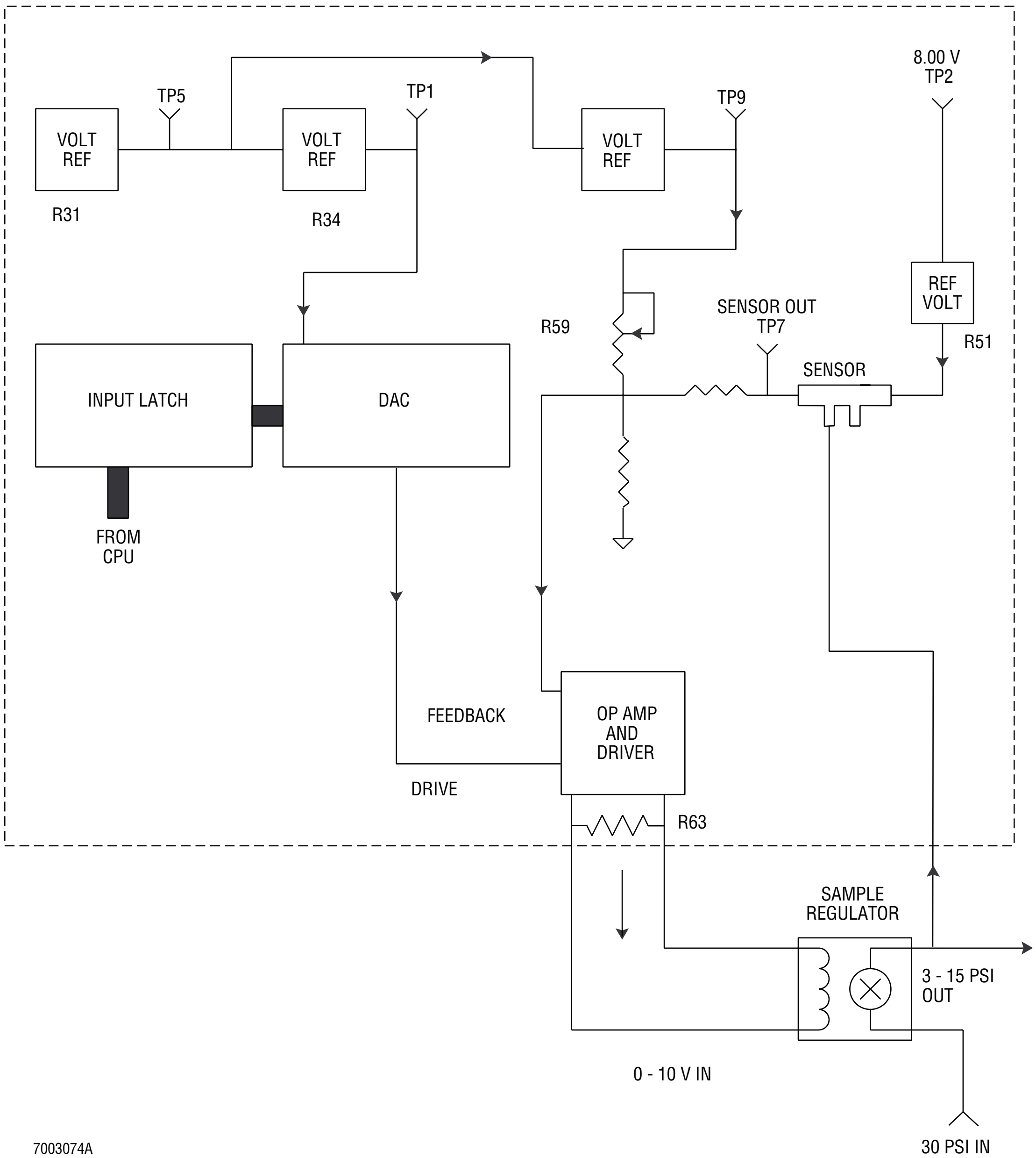
The system regulates sample pressure by comparing the DAC voltage that indicates the desired pressure with the voltage that the Sample Pressure Sensor circuit develops. After amplification, the difference, known as error voltage, drives the electronic pressure regulator.

R51 controls the power supply for the sample pressure sensor. This is nominally set to provide 8.00 V at TP2, which is the voltage input to the sensor. The sensor's output depends on this voltage and on the pressure input. You can monitor the sensor output at TP7.

Note: This is also the voltage the system applies to the Sensor Interface card for the readback display.

Although the sensor is linear, there is a 1 V offset from zero. The circuit compensates for this by summing a -1 V level with the sensor output to produce the feedback voltage applied to the op amp driver circuit for the regulator. You can adjust the offset compensation by R59. Therefore, R59 affects the Low Pressure circuit response more than it affects the High Pressure circuit response, and the DAC reference voltage (R34) affects the High Pressure circuit response more than it affects the Low Pressure circuit response. The adjustment procedure for the Pneumatic Interface card balances these two adjustments to obtain linear operation over the whole operating range.

Figure 2.9-2 Block Diagram, Sample Pressure System, Old Type



Regarding Figure 2.9-2:

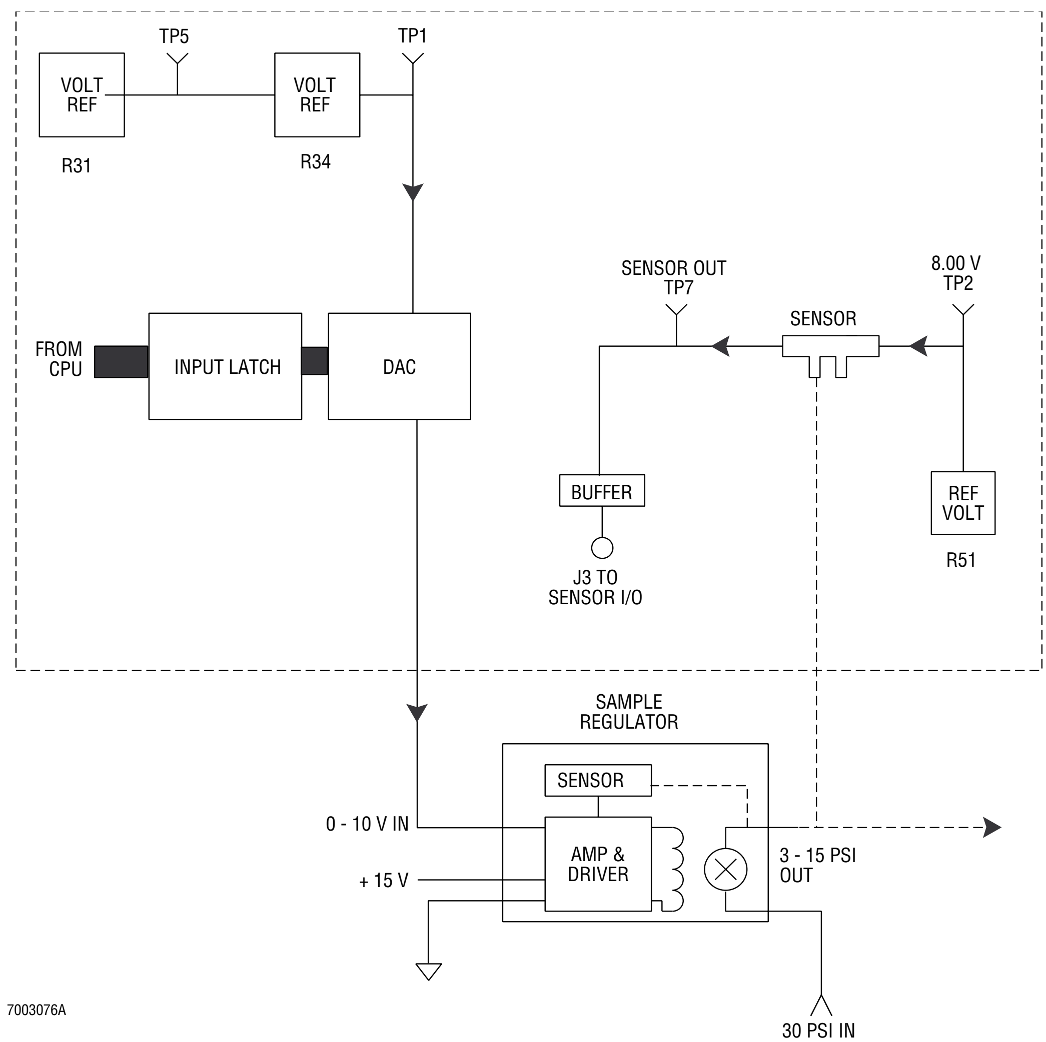
- The Input Latch is an 8-bit word, set pressure, written by CPU (U23).
- The DAC provides a voltage proportional to the digital pressure. Full scale is set by Volt Ref.

New Type

The new type of Sample Pressure System (Figure 2.9-3) consists of a DAC that converts the requested pressure in digital form to a 0 - 10 V analog level. This level goes to the low bleed sample regulator. The regulator uses an internal pressure transducer to sense and regulate the sample pressure.

A separate circuit consists of a pressure transducer, a reference power supply, and an amplifier to provide a voltage level to the Sensor Interface card indicating sample pressure. This is provided for display only and does not affect the sample pressure control circuit.

Figure 2.9-3 Block Diagram, Sample Pressure System, New Type



Regarding Figure 2.9-3:

- The Input Latch is an 8-bit word, set pressure, written by CPU (U23).
- The DAC provides a voltage proportional to the digital pressure. Full scale is set by Volt Ref.

Sheath Pressure Systems

There are two types of sheath pressure systems: old type and new type.

Old Type

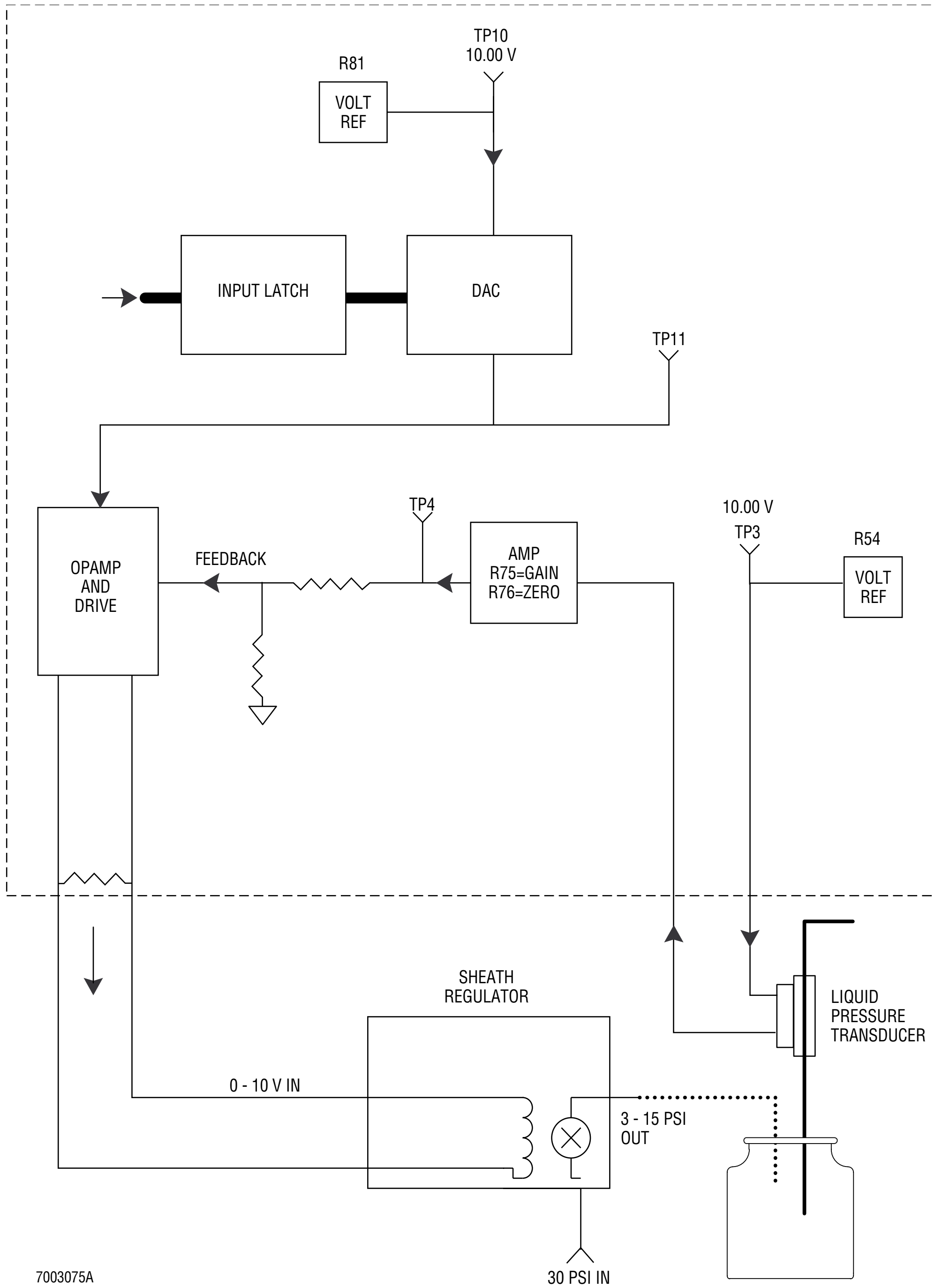
With the old type of Sheath Pressure system (Figure 2.9-4), the Sheath Pressure Regulation circuit is similar to the old type Sample Pressure circuit in that the CPU writes the desired pressure to the Pneumatic Interface card. The card compares the desired pressure with the pressure measured by the sheath pressure sensor. The Pneumatic Interface card then varies the voltage applied to the sheath pressure regulator to obtain the requested value.

This system differs from the Sample Pressure Regulation circuit in that the sheath pressure sensor actually measures the pressure present in the liquid line between the sheath tank and the flow cell. The reason for this is that the system must adjust the air pressure in the sheath tank to compensate for the change in liquid head pressure as the sheath level drops during operation. For this reason, you should make all adjustments when the sheath tank is full.

The sheath pressure sensor requires a 10 V power supply to operate. The R54 sets this requirement and you can measure it at TP3. Instrumentation-type Op Amp is interfaced to the sensor. This circuit provides both gain and offset adjustment for the sensor input. You can measure the output at TP4, which is the voltage provided for the Sensor Interface card for digitization and display on the screen as the “read” sheath pressure.

The CPU writes the desired sheath pressure to the Pneumatic Interface card. The DAC converts the information to an analog voltage. The value of the DAC reference voltage as measured at TP10 and adjusted by R81 determines the conversion scaling. The op amps that drive the sheath pressure regulator compare the DAC output with the amplified sensor feedback value to apply the appropriate voltage to the regulator to achieve the requested.

Figure 2.9-4 Block Diagram, Sheath Pressure System, Old Type



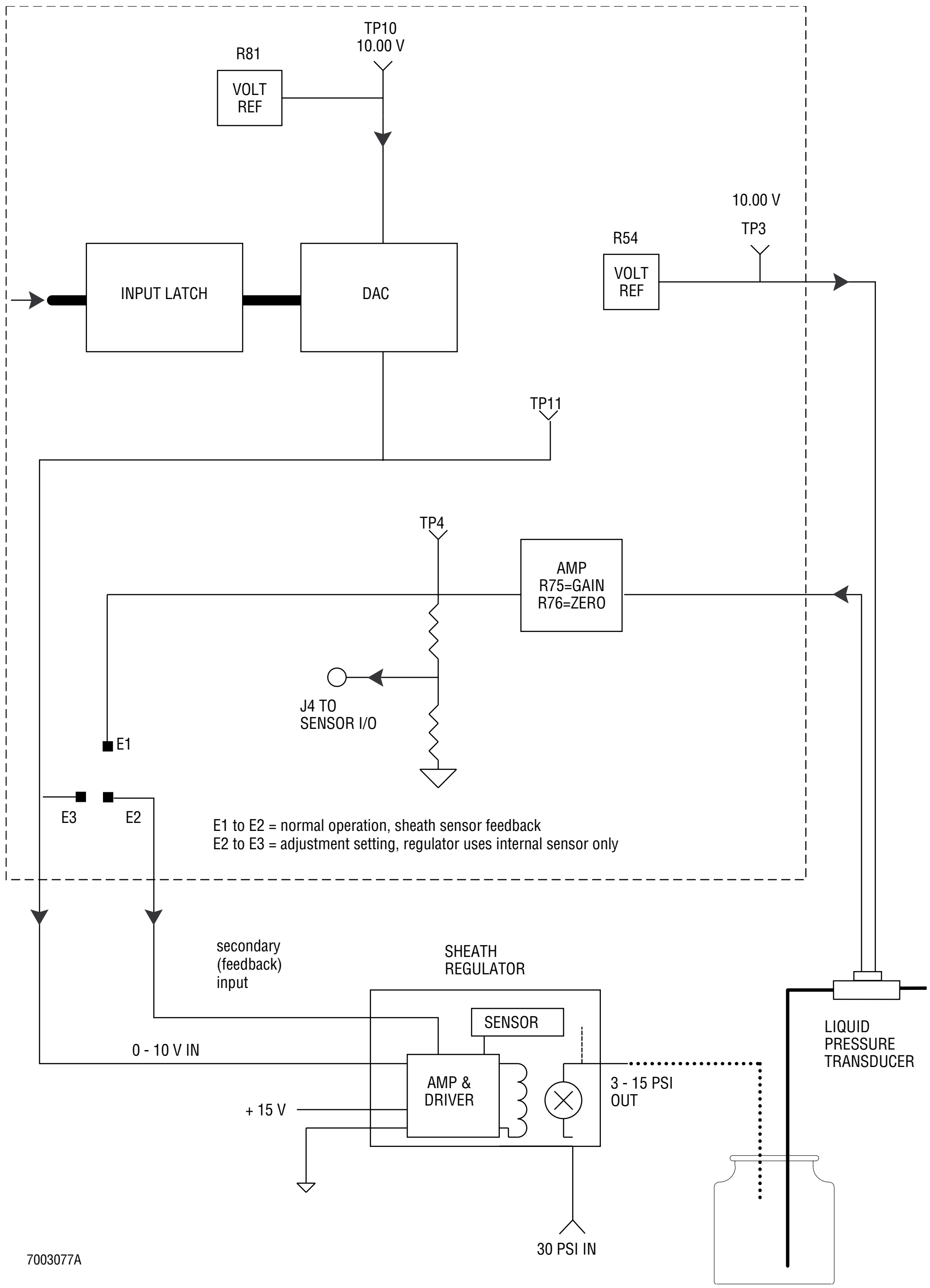
Regarding Figure 2.9-4:

- The Input Latch is an 8-bit word, set pressure, written by CPU (U17).
- The DAC provides a voltage proportional to the digital pressure. Full scale is set by Volt Ref.

New Type

With the new type of Sheath Pressure system (Figure 2.9-5), the circuit is slightly more complex than the Sample Pressure Regulation circuit because the system measures sheath pressure using a liquid pressure transducer at the output of the sheath tank. The amplified pressure transducer output goes to the Sensor Interface card for display on the Cytometer's Control screen. In normal operation, the amplified pressure transducer is connected to the pressure regulator to provide a feedback signal. The Pneumatic Control card can also be put in a test mode, where the feedback from the liquid pressure transducer is disabled, and the pressure regulator uses its internal air pressure sensor for feedback. In the test mode, the Pneumatic Control card does not compensate for sheath liquid level changes, which simplifies the card's adjustment and troubleshooting.

Figure 2.9-5 Block Diagram, Sheath Pressure System, New Type



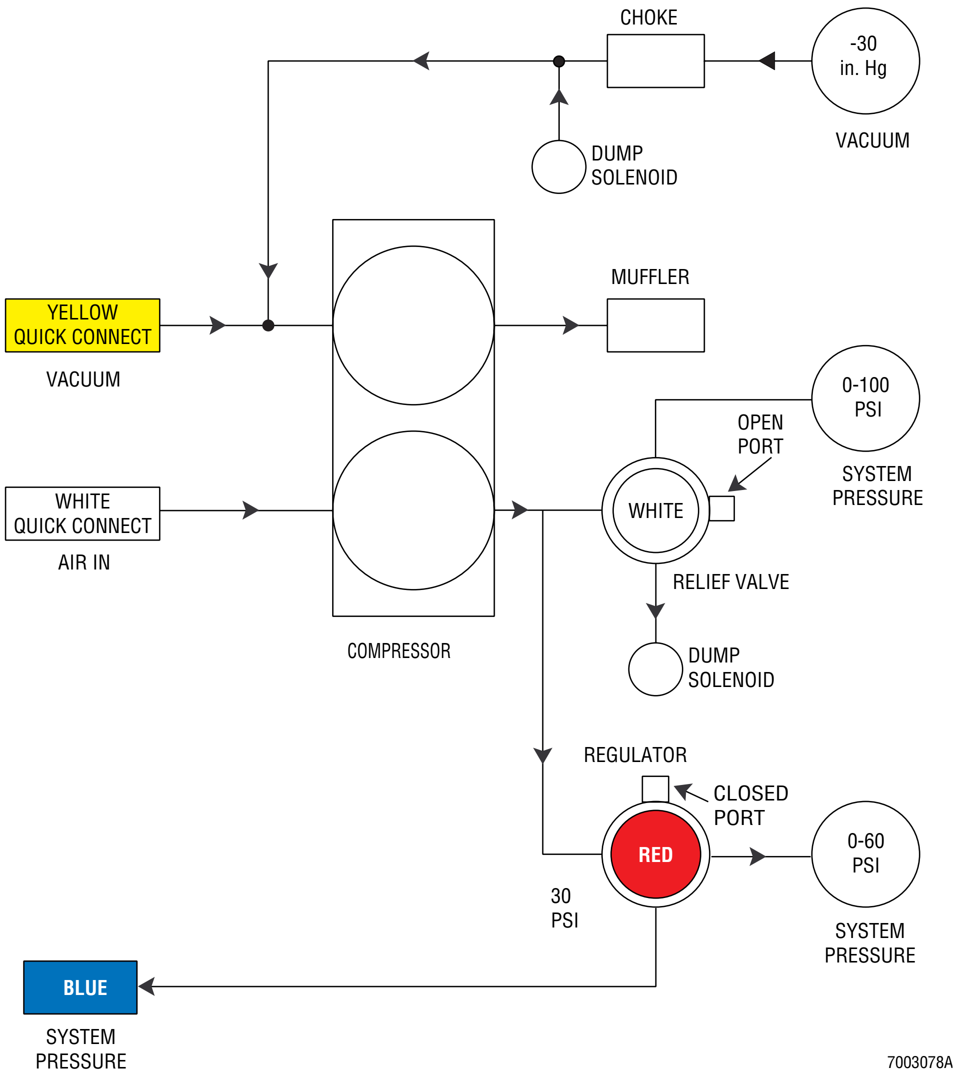
Regarding Figure 2.9-5:

- The Input Latch is an 8-bit word, set pressure, written by CPU (U17).
- The DAC provides a voltage proportional to the digital pressure. Full Scale is set by Volt Ref.

Compressor Module

The Compressor module (Figure 2.9-6) contains a single compressor with two heads and provides the system with pressure and vacuum. The module operates on 110 Vac 50/60 Hz. A transformer converts for different voltages.

Figure 2.9-6 Block Diagram, Compressor Module



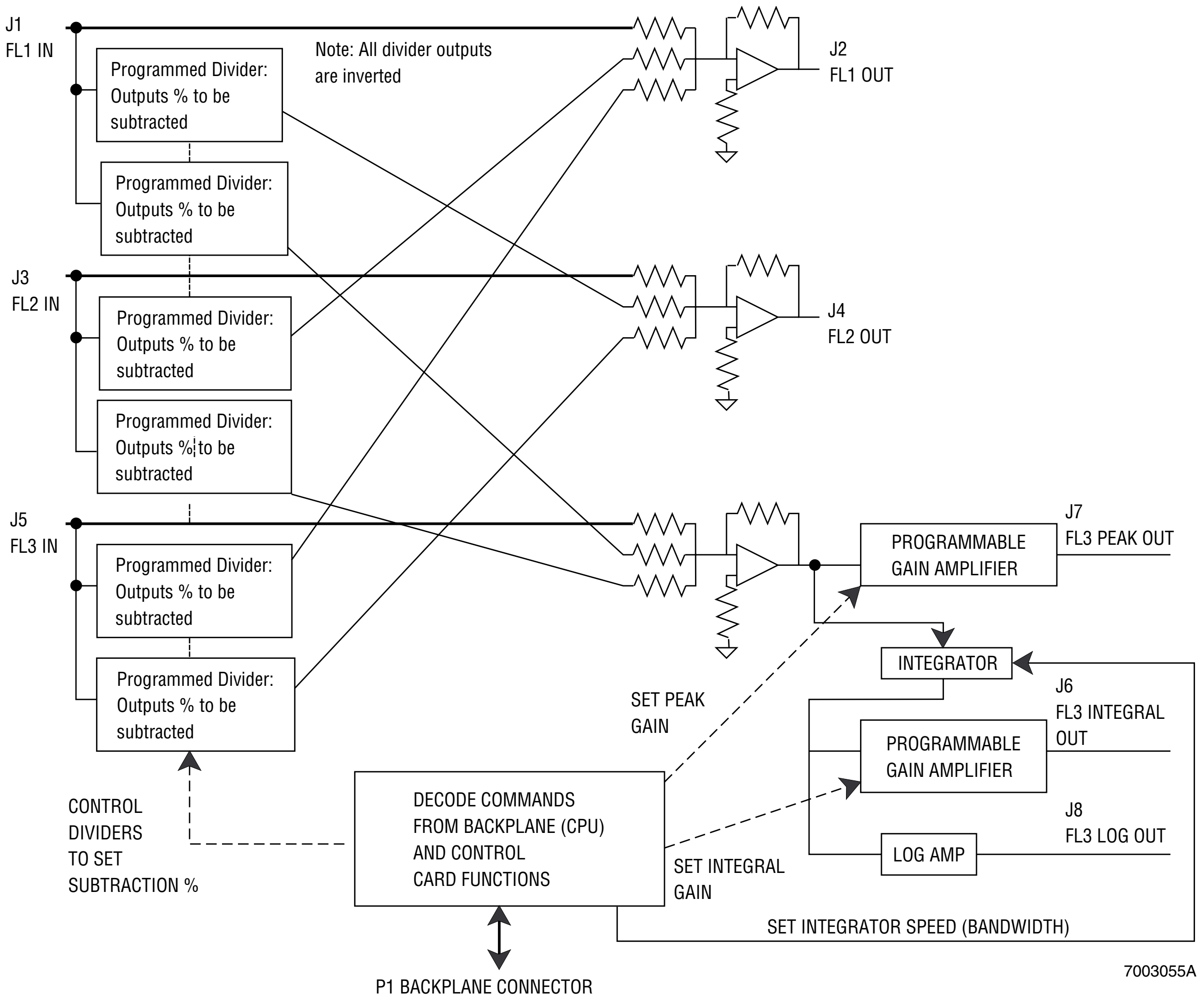
Mounting brackets secure the Compressor module for transport. However, for normal operation, the module must be set on the floor and cannot touch the instrument.

2.10 CARD DESCRIPTION - ALL CONFIGURATIONS

3 PMT Sub SW-R Card

The 3 PMT Sub SW-R card accepts three FL inputs. Each system can have a percentage of one or both of the other inputs subtracted from it. After subtraction, the card outputs the FL1 and FL2 signals. The card processes the FL3 signal to provide variable gain peak and integral signals and a log output. See Figure 2.10-1.

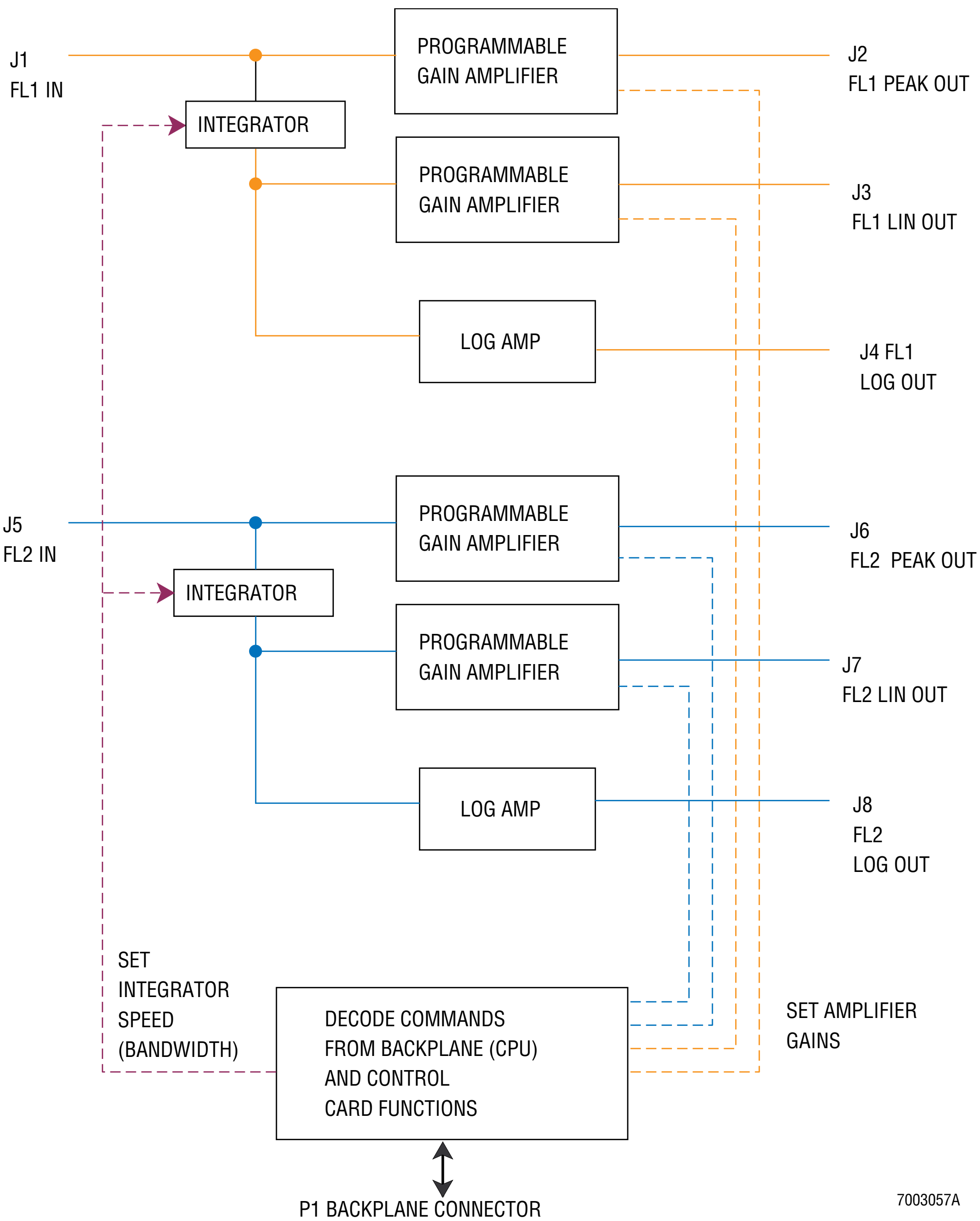
Figure 2.10-1 3 PMT Sub SW-R Card Block Diagram



Dual FL SW-R Card

The Dual FL SW-R card accepts two FL inputs and processes each input to provide variable gain peak and integral signals, as well as a log output. See Figure 2.10-2.

Figure 2.10-2 Dual FL SW-R Card Block Diagram

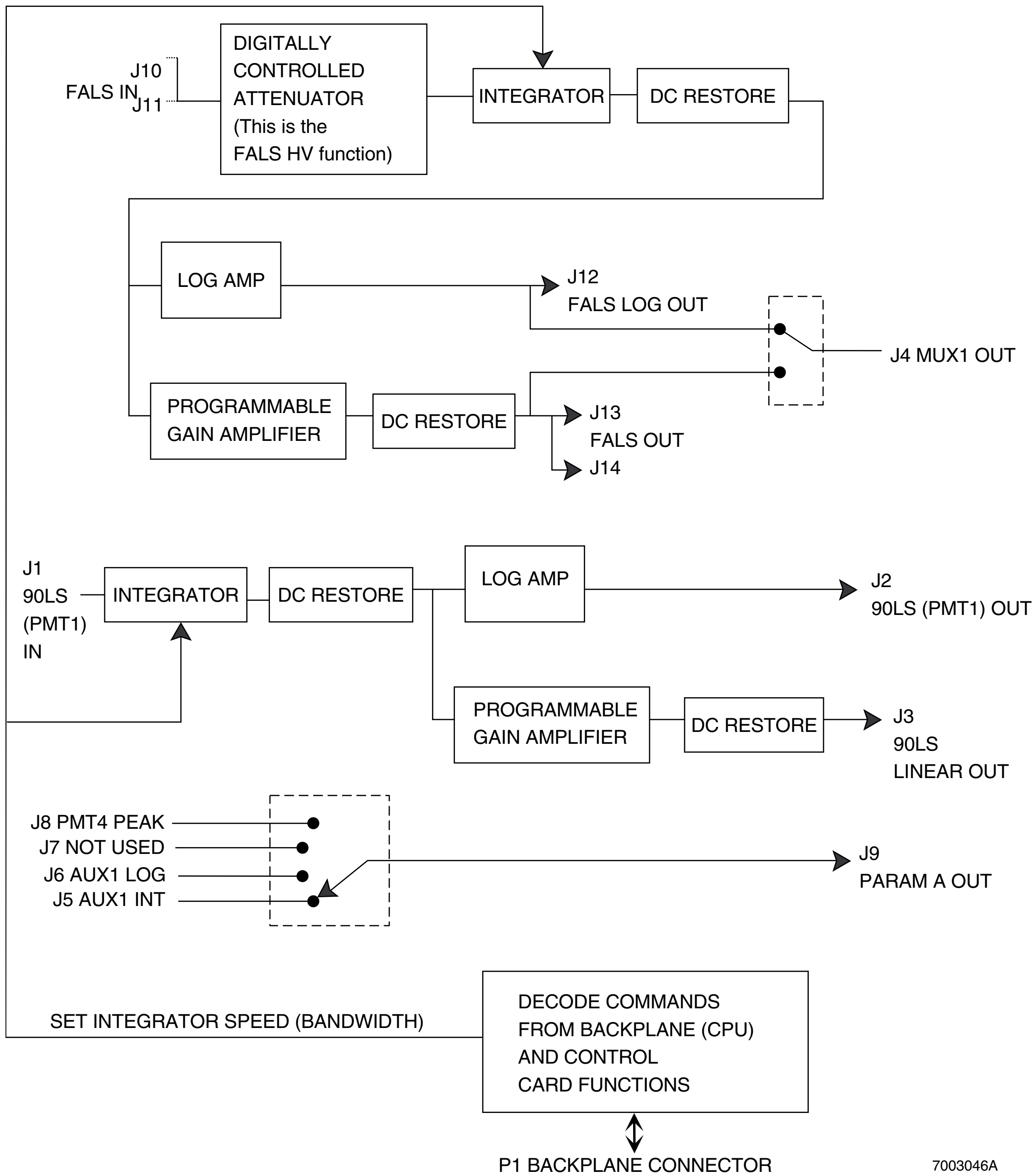


Scat/CV SW-R Card

The Scat/CV SW-R card accepts FALS and 90LS inputs. The FALS signal passes through a variable attenuator (FALS HV control) and is integrated. The card provides the FALS signal in parallel to a variable gain linear amplifier and a log amp. See Figure 2.10-3.

This card also incorporates two multiplexer circuits. The first Mux circuit selects either of the FALS signals as an additional output (J4). The second Mux, used only in a Gated Amp system, selects one of the four Mux inputs to go to J9.

Figure 2.10-3 Scat/CV SW-R Card Block Diagram

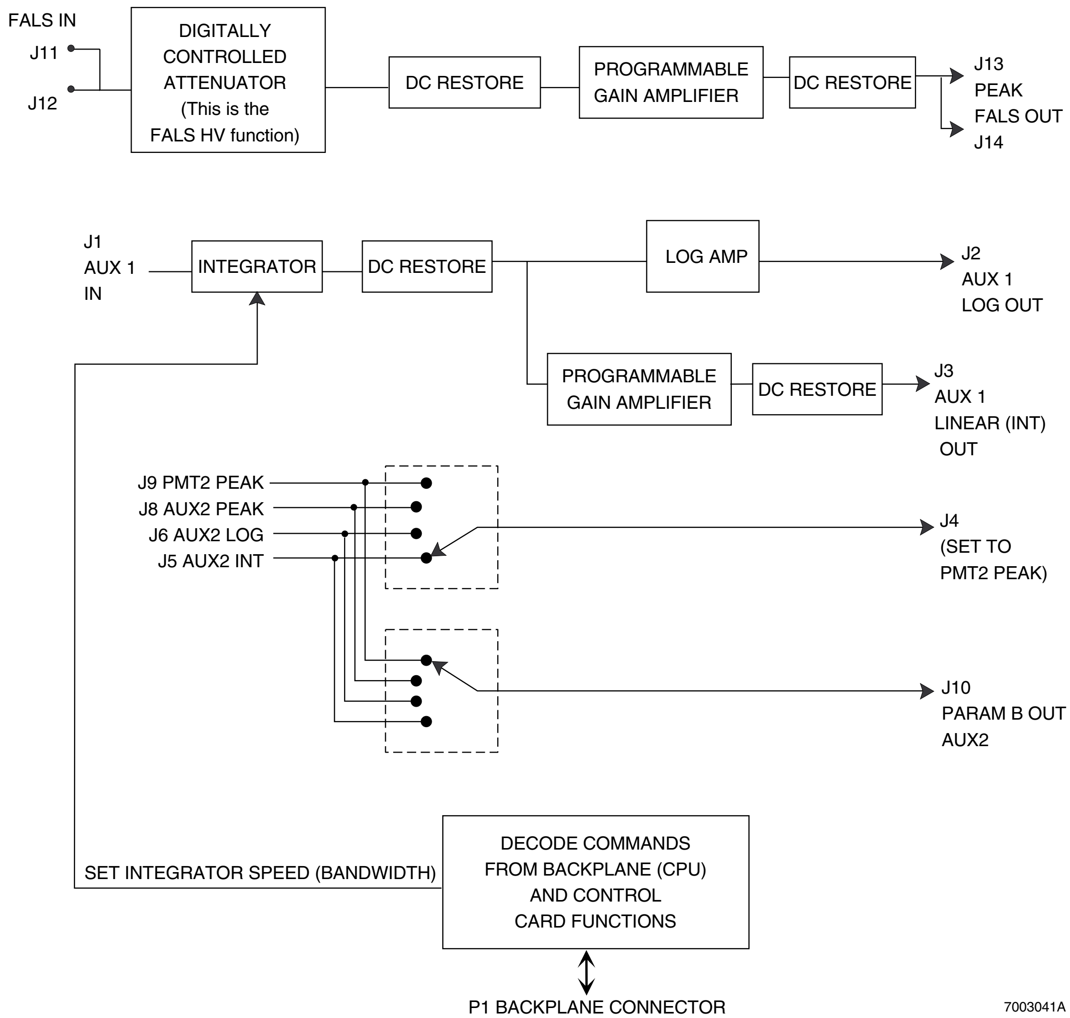


Peak Scatter Mux SW-R Card

The Peak Scatter Mux SW-R card accepts input from the Peak FALS detector and processes the signal to provide variable attenuator and programmable step gain control. The signal is not integrated. See Figure 2.10-4.

Additionally, this card accepts input from an auxiliary source (PMT). The card integrates the input and develops log and gain-controlled linear outputs.

Figure 2.10-4 Block Diagram, Peak Scatter Mux SW-R Card

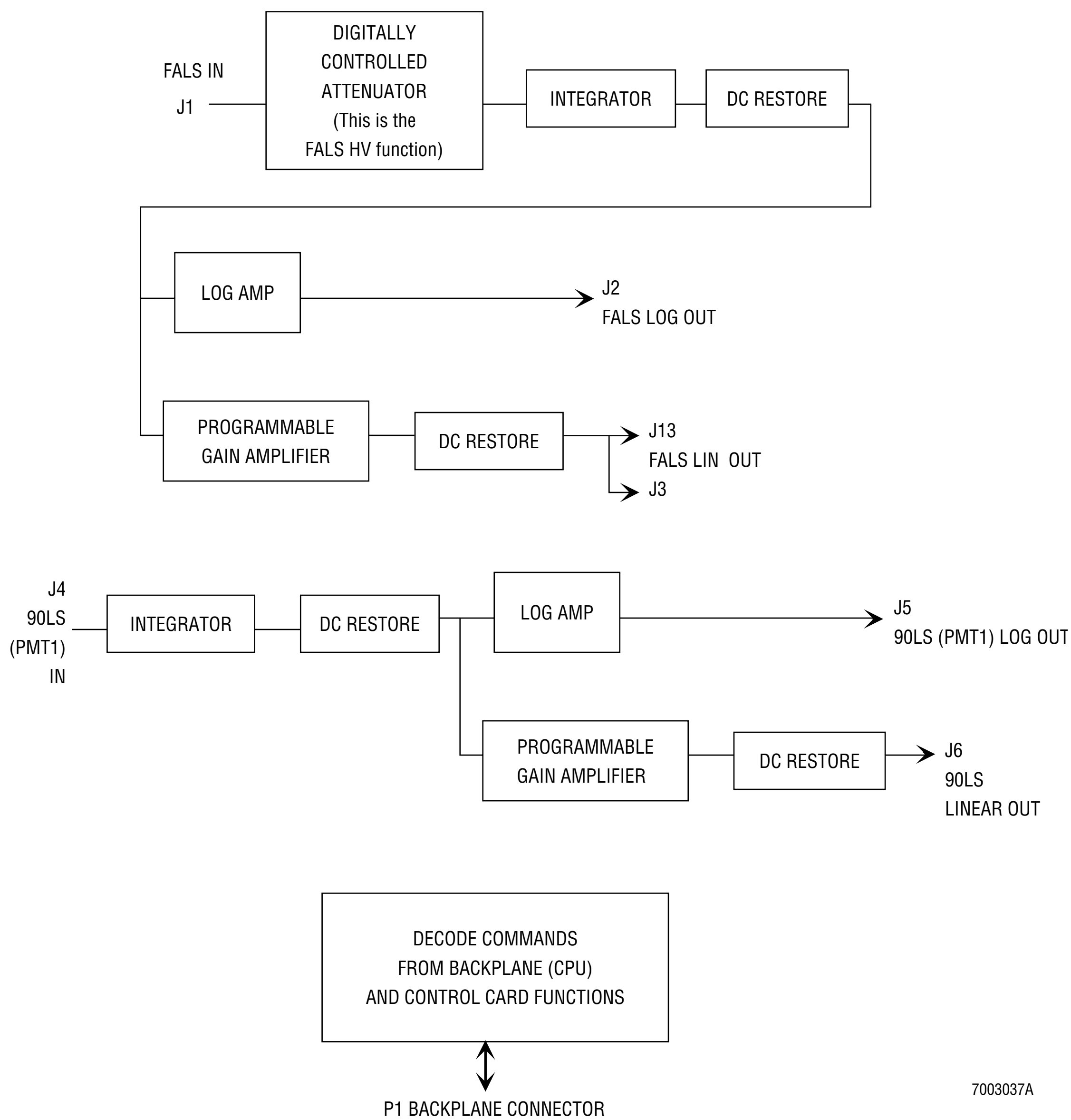


2.11 CARD DESCRIPTION - NON-GATED AMP NON-SWITCH CONFIGURATION (EXCEPT ANALYZER)

Scat/CV Amp Card

The Scat/CV Amp card accepts FALS and 90LS inputs. The FALS signal passes through a variable attenuator known as the FALS HV Control. This signal goes to a variable gain linear amplifier and a log amp. See Figure 2.11-1.

Figure 2.11-1 Block Diagram, Scat/CV Amp Card



3 PMT Sub Amp Card

Function

The 3 PMT Sub Amp card processes the FL3 signal to provide variable gain peak and integral signals and a log output. See Figure 2.11-2.

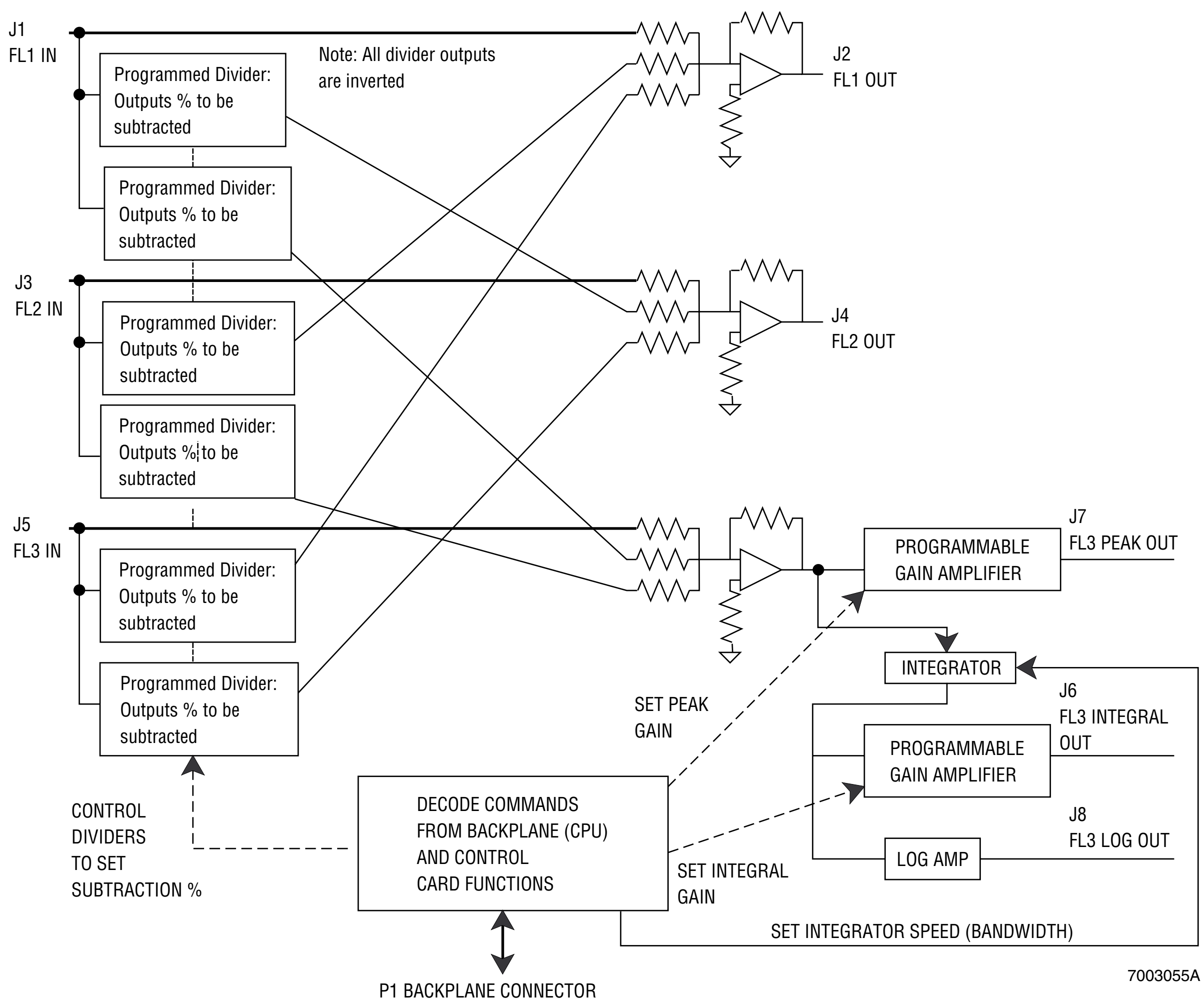
Jumpers

In Figure 2.11-2, the jumpers set the bandwidth for sense-in-air or quartz flow cells. All systems and cards are set to the quartz configuration as the default. For quartz operation, Elite 76 µ and 100 µ sort sense flow cells: X1, X2, X3, X4, X5, X6, X7, X8, X9, X0, E8-E9 = IN. For sense-in-air operation: X1, X2, X3, X4, X5, X6, X7, X8, X9, X10, E8-E9 = OUT.

Inputs

The 3 PMT Sub Amp card accepts three FL inputs, FL1, FL2, and FL3. Each input can have a percentage of one or both of the other inputs subtracted from it. After subtraction, the system outputs the FL1 and FL2 signals.

Figure 2.11-2 Block Diagram, 3 PMT Sub Amp Card



Dual FL Amp Card

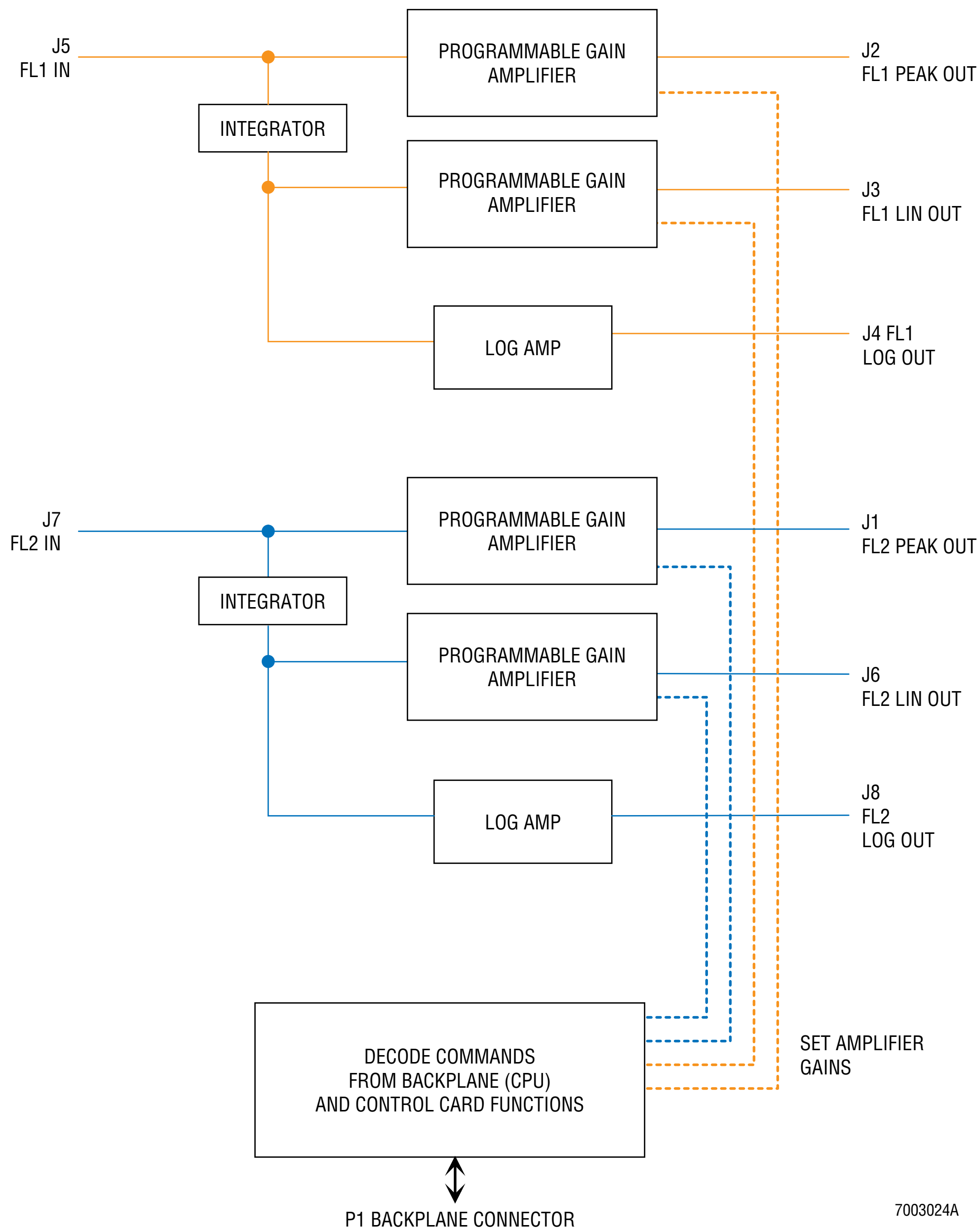
Function

The Dual FL Amp card processes each input to provide variable gain peak and integral signals, as well as a log output. See Figure 2.11-3.

Inputs

The Dual FL Amp card accepts two FL inputs, FL1 and FL2.

Figure 2.11-3 Block Diagram, Dual FL Amp Card



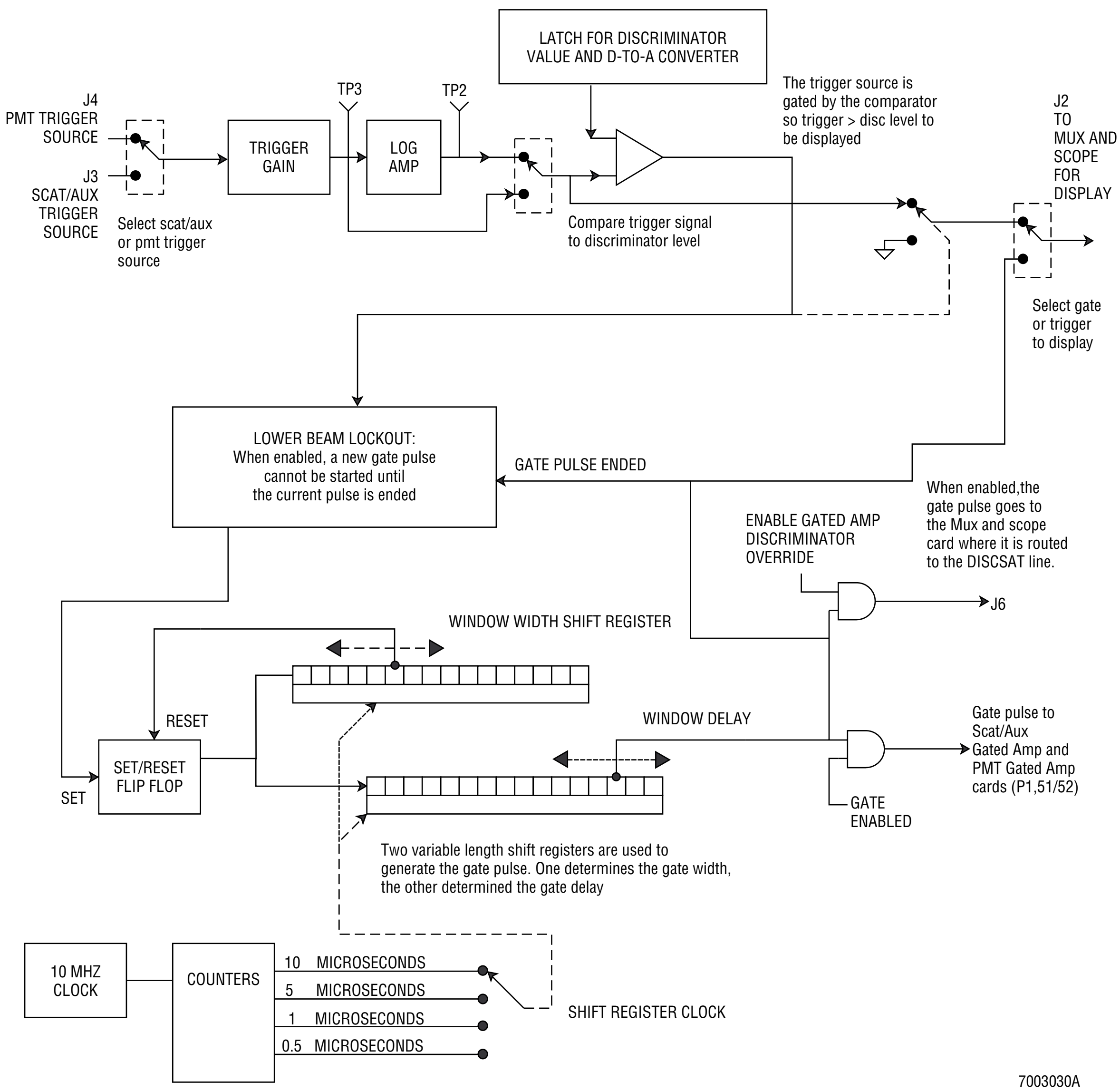
DUAL FL AMP, NON SWITCHAMPS

2.12 CARD DESCRIPTION - GATED AMPLIFIER CONFIGURATION

Gated Amp Control Card

The Gated Amp Control card provides an interface and cable connection between the DT Interface card and the Gated Amp backplane (upper backplane). The card recognizes the range of port addresses that correspond to cards in the upper backplane and puts the proper addresses and data words on the backplane. See Figure 2.12-1.

Figure 2.12-1 Block Diagram, Gated Amp Control Card



Gated Amp R3 Card

All Gated Amp configurations require the Gated Amp Control R3 card.

Functions

The Gated Amp Control R3 card:

- Selects the trigger source between the input from the Scat/Aux Gated Amp card or input from the PMT Gated Amp card
- Attenuates the trigger source via a programmable attentuator; then passes the trigger through a log amp. The amplified or unamplified (linear) signal is then selected as the trigger
- Compares the selected trigger signal to a programmed dc level (trigger level) and activates the gate window generator if the trigger level is exceeded. The generator is based on two timing circuits. The first provides a time delay starting from when the trigger level is exceeded. The time delay is the window delay period. After the window delay period ends, a second timing circuit generates a programmed length pulse, which is the window width pulse. When enabled, this pulse is routed to the Gated Amp card which then gates the actual signals.
- Selects the window pulse (gate window) or the trigger source to send to the Mux and Scope card for display.
- Intercepts the command to select which channels are to be delayed. This command is decoded and used to toggle individual lines on the backplane that go to the Gated Amp card(s), which actually selects the delays.
- Prevents retriggering of the timing circuits until the current timing cycle is completed when the Lower Beam Lockout is selected.
- When the Gate Window Discriminator Override is selected, the gate window is routed to the Mux and Scope card where it is connected to the DISCSAT line, forcing the Gated Amp Discriminator Level to become the System Discriminator.

Scat/Aux Gated Amp Card

Functions

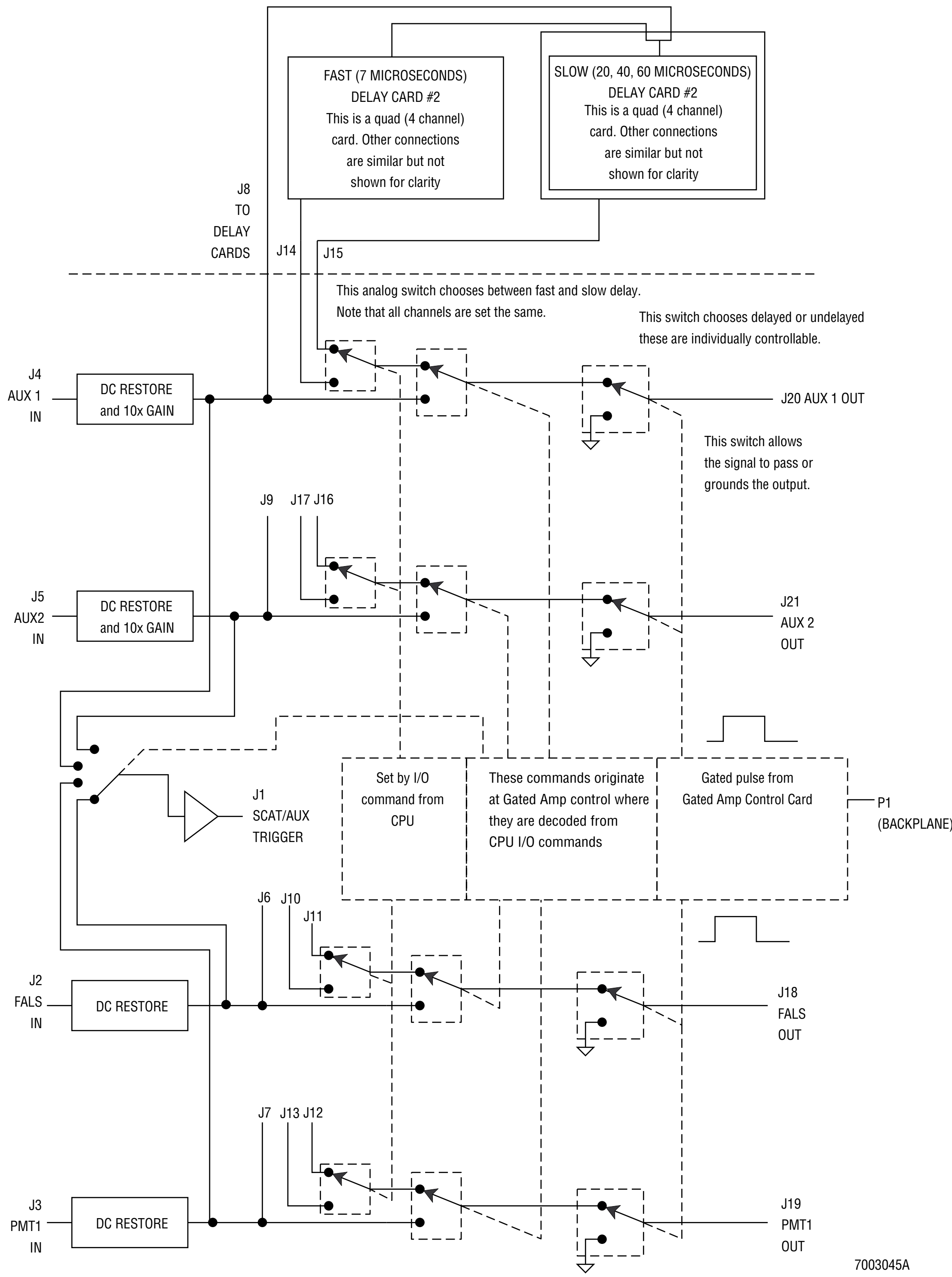
The Scat/Aux Gated Amp card (Figure 2.12-2):

- Selects one of the four signals to be the trigger source and routes the signal to the Gated Amp Control card.
- Sends all four signals to the delay cards. For each channel, the card uses the returned delayed signal or the undelayed signal.
- Gates by switching the signal outputs between the signal inputs or ground. The gate pulse from the Gated Amp Control card controls the gating.

Inputs

The Scat/Aux Gated Amp card has four signal inputs: FALS, PMT1 (90LS), Aux1, and Aux2.

Figure 2.12-2 Block Diagram, Scat/Aux Gated Amp Card



PMT Gated Amp Card

Function

The PMT Gated Amp card (Figure 2.12-3):

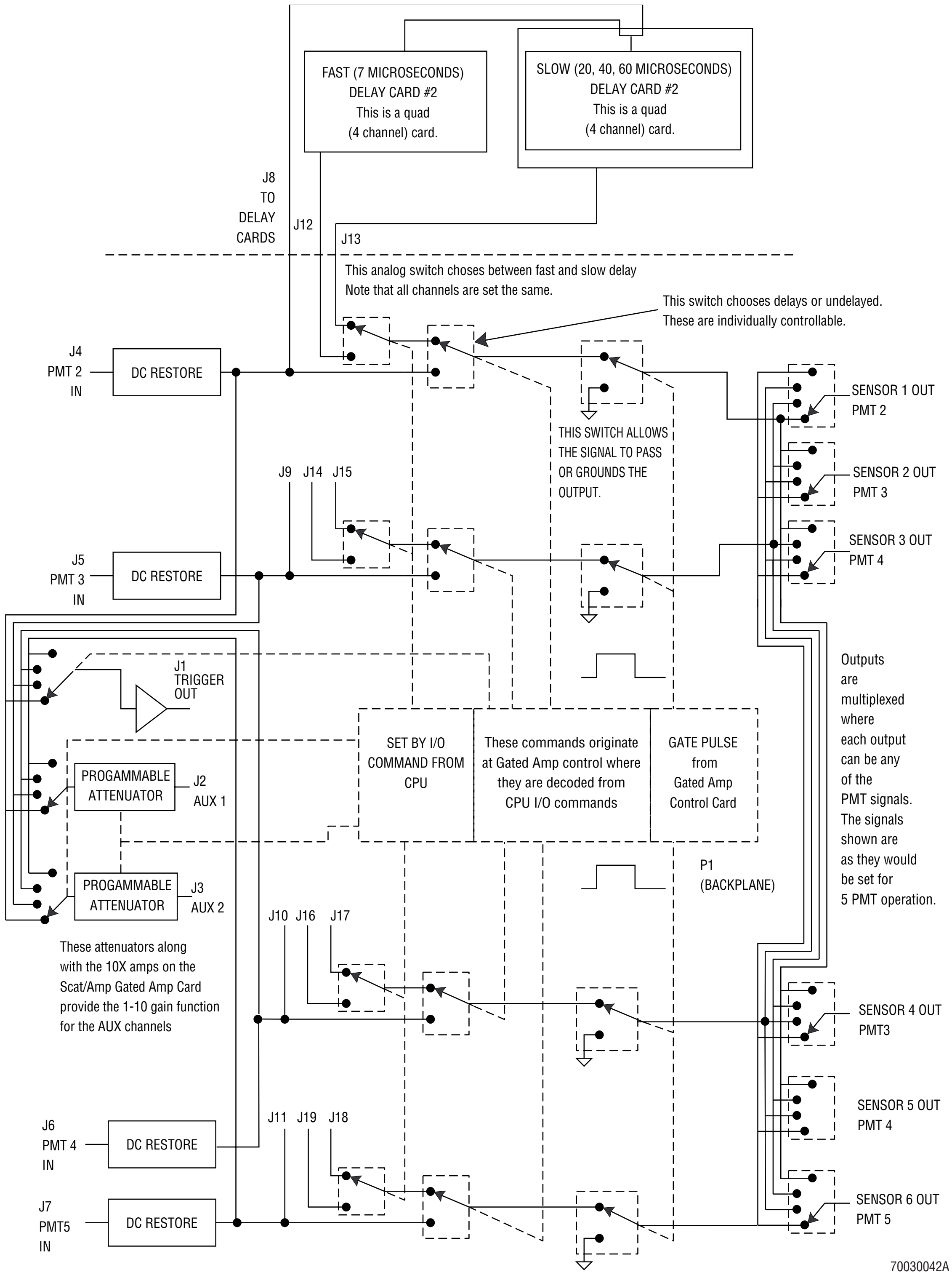
- Selects one of the four signal inputs to be the trigger source and routes it to the Gated Amp Control card.
- Selects two signals to be AUX1 and AUX2 from the four signal inputs and sends the signals to the Scat/Aux Gated Amp card.
- Sends the four input signals to the delay cards. For each channel, the PMT Gated Amp card can use the returned delayed signal or the undelayed signal.
- Gates by switching the signal outputs between the signal inputs or ground. The gate pulse from the Gated Amp Control card controls the gating.

Inputs

The four signal inputs for this card are:

- PMT2
- PMT3
- PMT4
- PMT5.

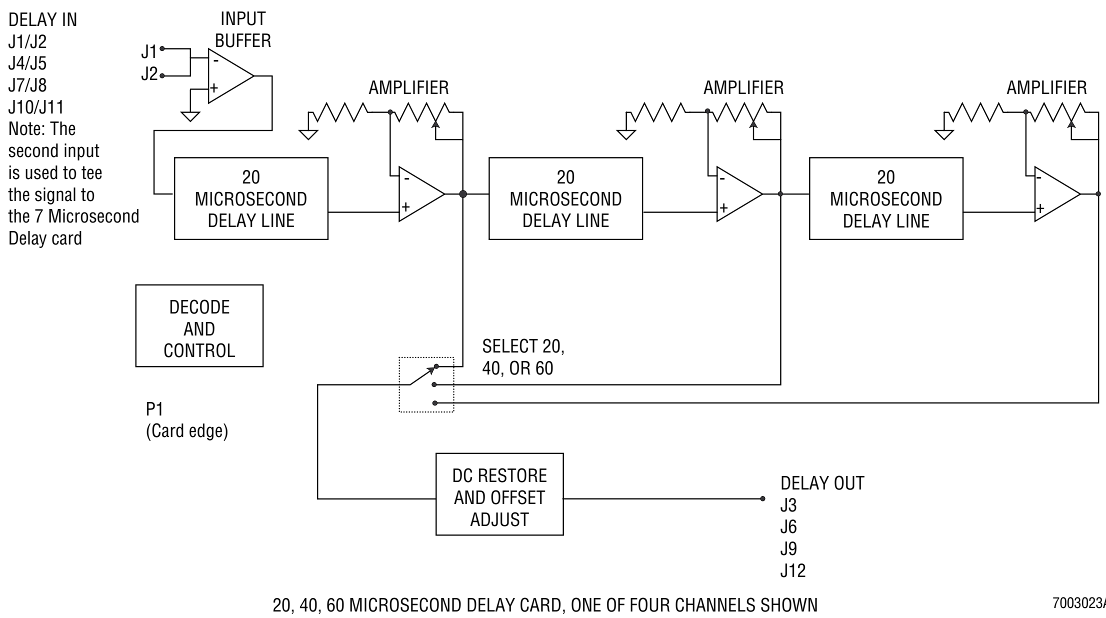
Figure 2.12-3 Block Diagram, PMT Gated Amp Card



20/40/60 Microsecond Delay Card

The 20/40/60 Microsecond Delay card delays the incoming pulse for a selectable delay of 20, 40, or 60 microseconds by selecting one, two, or three cascaded 20-microsecond delay lines. The gain of each 20 microsecond delay section is individually adjustable to ensure unity gain. See Figure 2.12-4

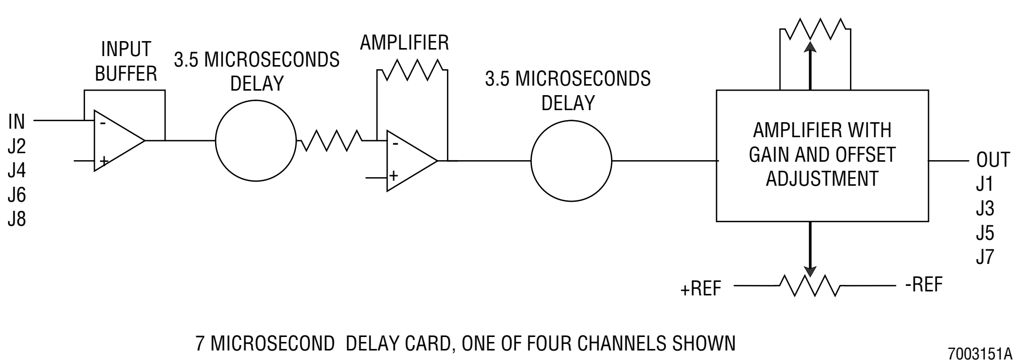
Figure 2.12-4 Block Diagram, 20/40/60 Microsecond Delay Card



7 Microsecond Delay Card

The 7 Microsecond Delay card delays the incoming pulse for seven microseconds. Output gain and offset are adjustable. See Figure 2.12-5.

Figure 2.12-5 Block Diagram, 7 Microsecond Delay Card



2.13 SIGNAL SELECTION AND DIGITIZATION

Overview

Before considering the functions and operation of individual circuit cards, you must first understand how the cards work together as a system to acquire data.

Each cell or particle that the Elite processes is called an event. And each event represents one or more pulses containing different information about the same cell. The Elite must process these pulses separately but retain the fact that the parameters are for the same cell. Visualize the system as operating on an event-by-event basis or cycle.

The acquisition cycle begins when at least one channel senses a cell's presence. The system stretches and holds all pulses for the event. The ADC converts stretched pulses to digital values and sequentially places theses values on the data path bus. At this time, a frame of sequential values on the data path bus represents the event. The following can read the digital values as they appear on the bus: Prism and Sort Window Test card, Bitmap and Sort Decision card, and Data Lister Out card.

The Multibus CPU card writes values to the registers of the cards used in acquisition, determining how they will function during acquisition. The CPU has nothing to do with the acquisition cycle except to turn the acquisition process on and off.

The sensors simultaneously measure each cell that passes through the laser. The Elite correlates the data that comes from the sensors. The value of 0 is a valid piece of information, because is means that the particular cell had no fluorescence or no light scatter. It is important that the voltage value from a particular parameter remains associated with that particular cell.

To achieve correlation, the Elite uses one channel to detect the presence of cells in the laser beam and then commands all other channels to measure what they "see" at that time. This process is called forced conversion.

The system must compensate for slight timing differences that occur for different types of parameters. The ADC and PSH Control card works with the PSH (Peak Sense and Hold) cards to perform the compensation.

The discriminator circuit monitors the pulse entering the PSH card. This circuit also determines if a particular pulse (between 0 and 10 V) exceeds the discriminator level, which can be set between 0 and 10 V.

If the discriminator level is exceeded, the ADC directs the PSH cards to capture and hold the pulses that are present at that time. To accomplish this, the ADC controls the OPEN signal to the PSH cards; this creates an acquisition window within which the system accepts the data. The length of the window depends upon the type of ADC the system uses, the discriminator level, the acquisition start delay setting, and the pulse(s) that trigger the discriminator. Refer to the ADC and PSH Control Card under Signal Flow Description for more information.

Signal Flow Description

Signal Source

All signals entering Mux and Scope card have been properly amplified and processed for display, acquisition, and sorting by the cards described in Overview under Heading 2.13, SIGNAL SELECTION AND DIGITIZATION.

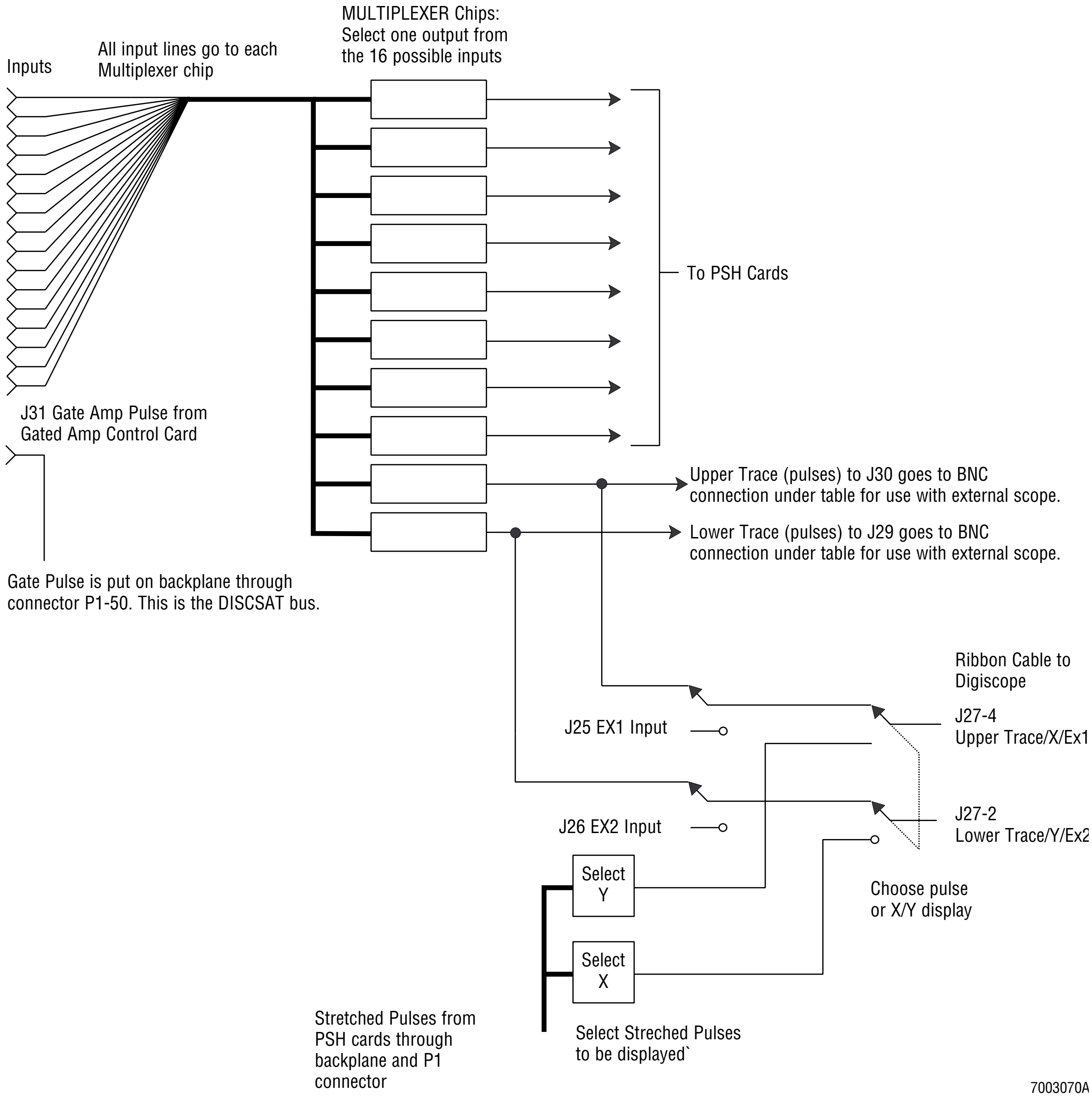
Mux and Scope Card

The Mux and Scope card (Figure 2.13-1):

- Selects up to eight signals for acquisition or sorting. The coax cables carry the signals to the two Peak Sense and Hold (PSH) cards.
- Selects two signals for pulse display. A ribbon cable takes the signals to the Digiscope card for possible display.
- Receives stretched pulses from the outputs of the PSH cards. The card can select two of these pulses for possible scattergram display.
- Is controlled by the Multibus CPU card.

For Gated Amp systems, two additional inputs are used. One allows display of the Gate Window or Gated Amp Trigger pulse for adjustment and alignment purposes. The second connection provides the Gated Amp card access to the DISCSAT bus to implement the Gate Amp Discriminator Override function.

Figure 2.13-1 Block Diagram, Mux and Scope Card



Quad Peak Sense and Hold (PSH) Cards (2)

Together, two Quad PSH cards (Figure 2.13-2) provide eight parallel acquisition channels. Each channel's purpose is to develop a voltage output that represents the maximum value (peak) of the input pulse. This voltage output is held by the Peak Sense and Hold circuit until the conversation cycle is completed and the card receives a reset command. The discriminator circuitry determines which pulses the card captures and holds.

Each Quad PSH card is organized into four identical channels. The system traces the signal flow through one channel. The card:

- Limits incoming signal to 10 V to ensure that all off-scale pulses are placed in the last channel of the histogram.

- Implements the discriminator function of the system.

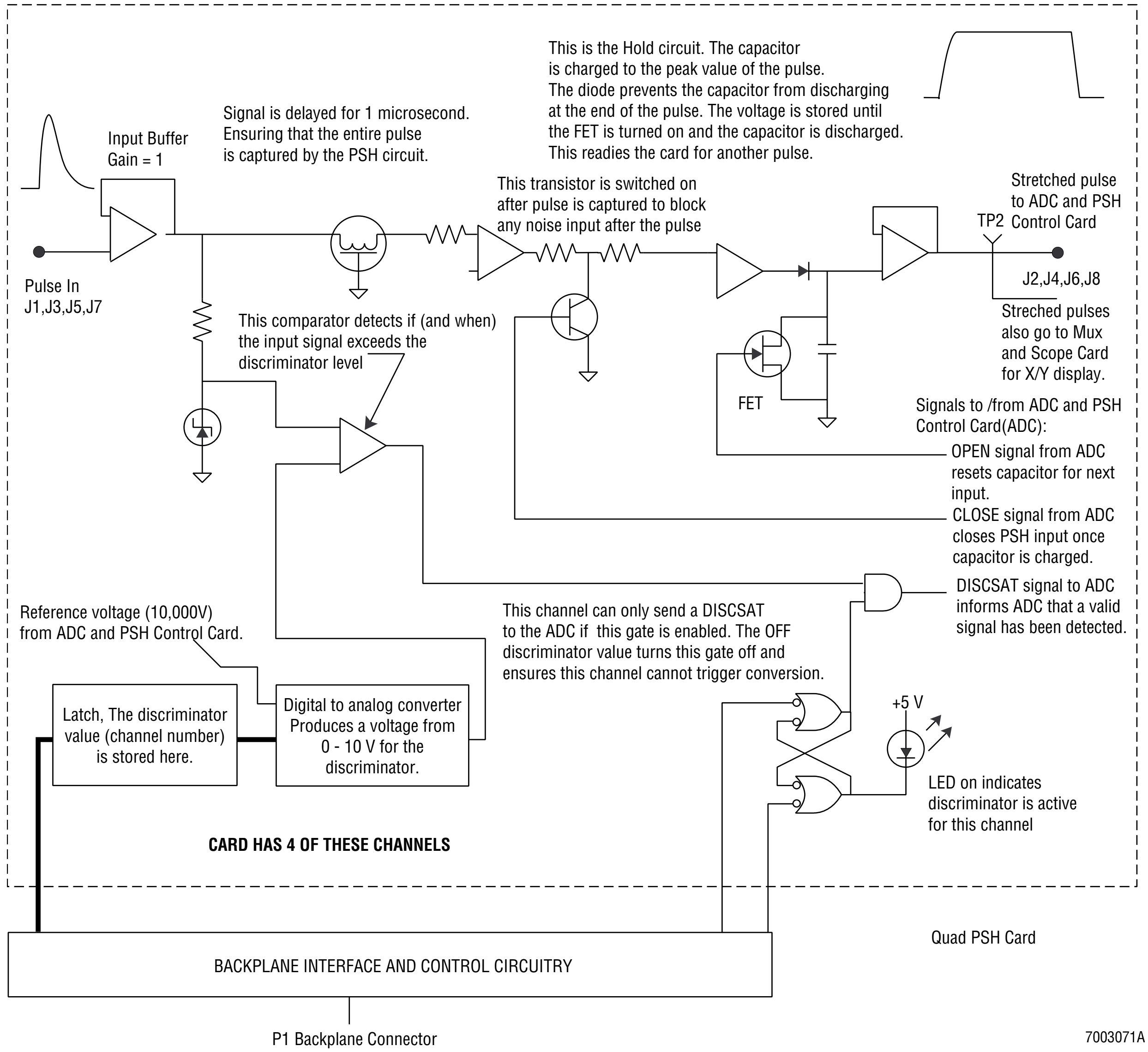
  Before acquisition, the system loads a discriminator value into a latch on the card. A digital-to-analog converter (DAC) converts the latch output to an analog voltage and provides one input to a voltage comparator. The input pulses are provided to the other comparator input. This comparator's output is gated by whether the particular PSH channel is active. This condition is set if a discriminator value is set to this channel (including channel 1023); if this channel is set to OFF, then the output is blocked.

  The card uses an open collector gate that permits all of these outputs to share a common line to the ADC and PSH Control card. This common line is called DISCSAT and is normally pulled high to 5 V through a resistor. Therefore, when any discriminator on any PSH channel is exceeded, the DISCSAT line is pulled low. This signals the ADC and PSH Control card that a cell is present and that data needs to be acquired.

- Captures and holds the highest voltage value of the incoming pulse.

  The Peak Sense and Hold circuit performs this function. The signal pulse can charge a capacitor through a diode. This ensures that the capacitor is charged to the highest (peak) value of the incoming pulse, and prevents the capacitor from discharging when the pulse ends. The capacitor holds the charge until the ADC and PSH Control card sends a discharge signal back to the capacitor. The pulse that leaves the card is the stretched pulse. The PSH circuit's input contains a switch-to-ground that the ADC and PSH Control card controls. This isolates the card's input from noise after a valid pulse is captured.

Figure 2.13-2 Block Diagram, PSH Card(s)



ADC and PSH Control Card

The ADC and PSH Control card (Figures 2.13-3 and 2.13-4) contains an Analog Calculator circuit that can calculate the ratio of any two inputs. The voltage that represents this ratio is routed out of the card on J17 and is jumpered back into J9, where it is treated as any other incoming parameter.

Figure 2.13-3 Block Diagram, ADC and PSH Control Card

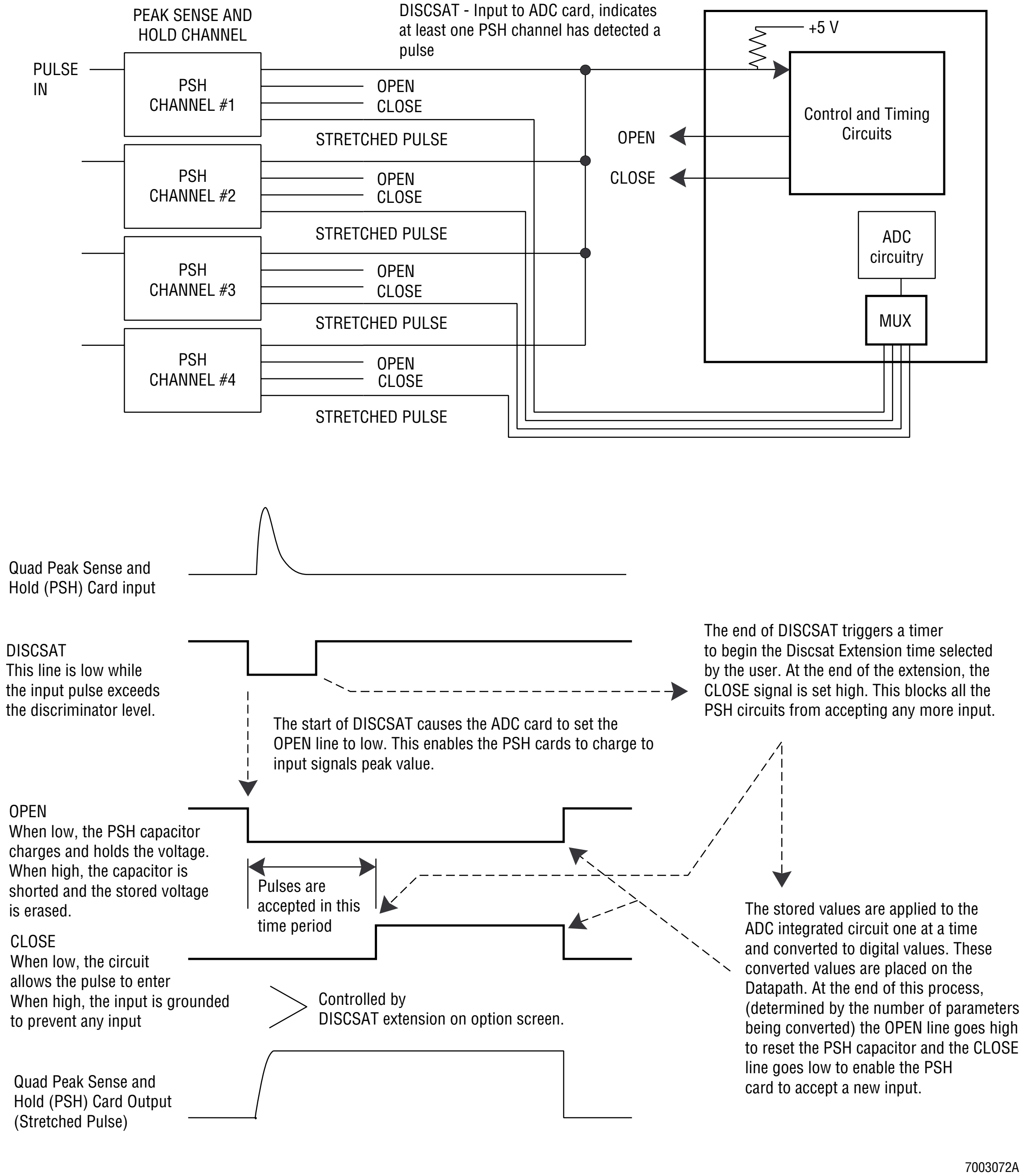
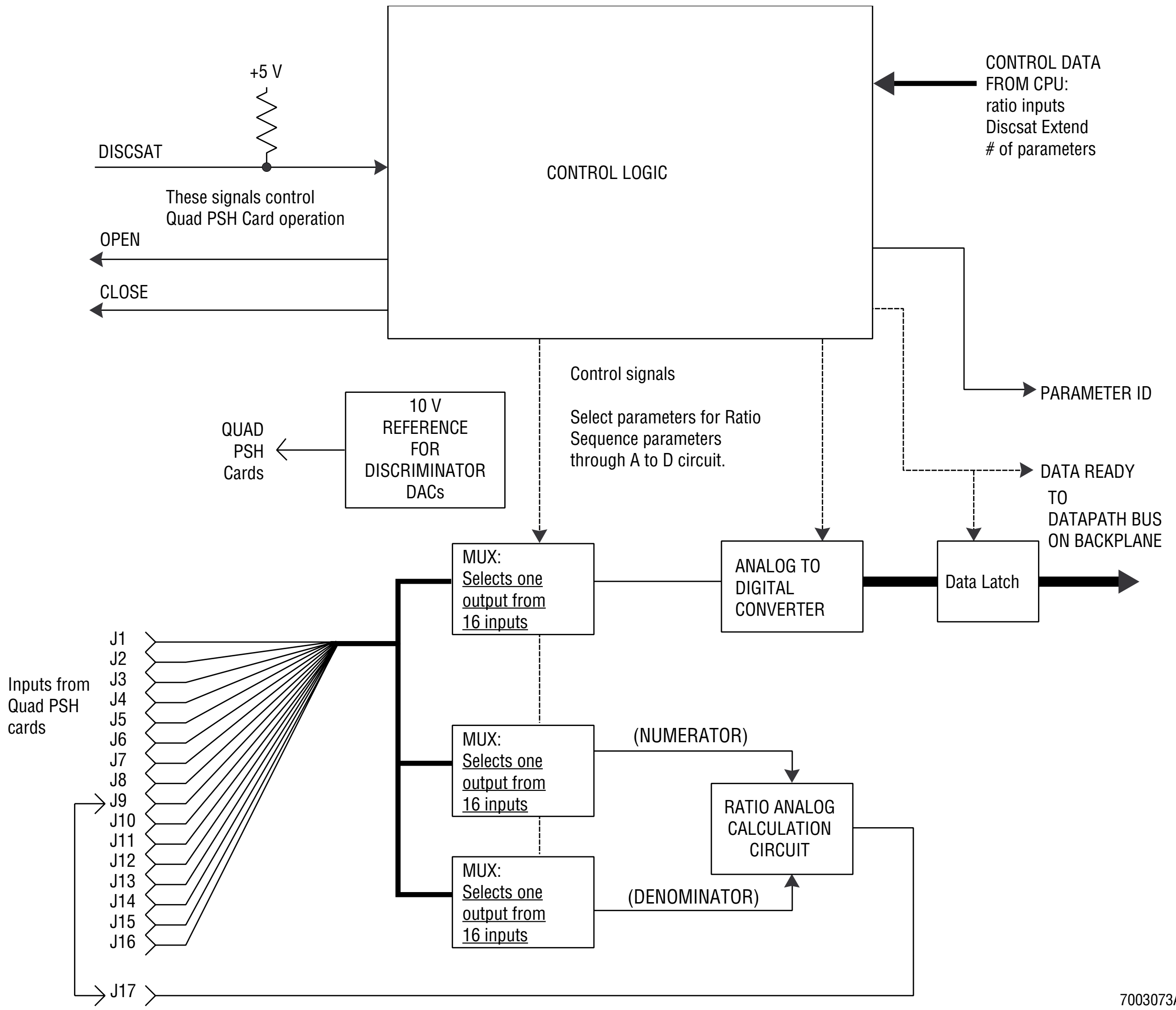


Figure 2.13-4 Block Diagram, Peak ADC PSH Control Card



As shown in Figure 2.13-4, each DISCSAT triggers a timing cycle that, for each event:

- Operates the PSH cards to capture analog data for each parameter using the specified DISCSAT Extension to control the acquisition window
- Sequences the analog data from each active parameter to the A to D circuit
- Sequentially places the converted (digital) values on the backplane
- Resets the PSH cards for the next event.

The ADC and PSH Control card:

- Is configured by the CPU, prior to acquisition, to process the correct number of parameters and use the specified timing parameters.

  Once acquisition begins, the card cycles through the acquisition process until it receives a command to stop acquisition.
- Controls the acquisition cycle.

  When a PSH card indicates a discriminator satisfied condition, then the cycle starts.

Note: In a Gated Amp system, this can also originate from the Gated Window pulse.

- The ADC and PSH Control card allows all active PSH cards to charge to the maximum input level, then closes the PSH inputs to prevent corruption of the stored values. Refer to ADC and PSH Timing for details.
- The ADC and PSH Control card receives analog data (stretch pulses) from the PSH cards and digitizes these inputs one at a time, and sequentially places the digital values on the data path bus.

When the cycle is completed, (depending on the number of pars being converted) the PSH storage capacitors discharge and the PSH inputs reopen to prepare for the next event.

ADC and PSH Timing

The Elite uses two different ADC and PSH Control cards as listed in the following chart.

System	Date of System Manufacture	Card Used	ASD Operation Supported by Card
Elite	Before 09/93	ADC and PSH Control	No
Elite ESP	09/93 and after, or any field-upgraded system	Peak ADC	Yes

The cards differ in their implementation of Acquisition Start Delay (ASD), also called discriminator extension.

ASD allows the operator to control the length of time that the acquisition system samples the signals from each cell. The length of time must be properly set, otherwise, problems could result. The time must be long enough to allows all signals sufficient time to reach their maximum values, yet short enough to avoid noise or signal contamination from other particles.

When determining and setting an optimum time, consider:

- Hardware factors -
  - Flow cell type
  - Laser beam size
  - Amplifier operating mode (fast or slow).
- Protocol requirements -
  - Types of signals being acquired (peak, integral, or log)
  - Which signal is used for the discriminator channel.

Changes in any of the above factors may require you to change the ASD.

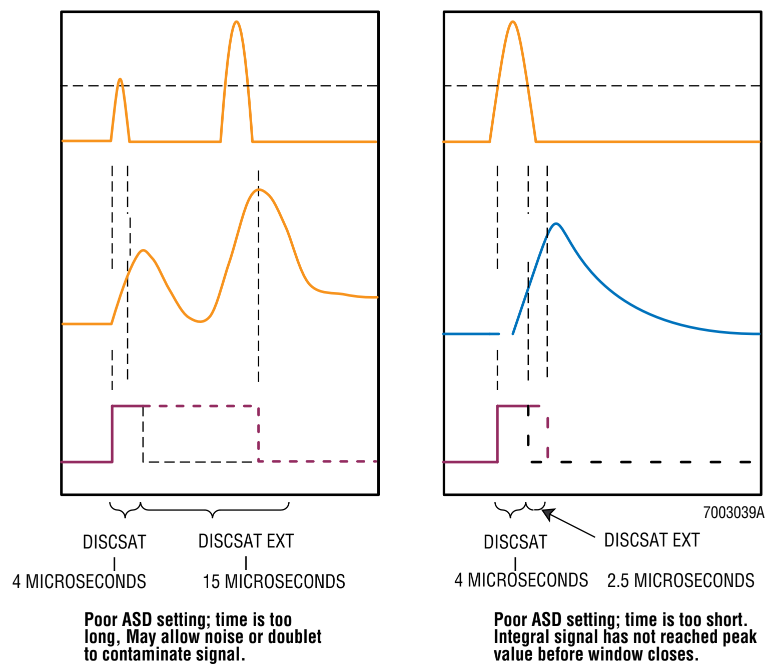
Note: The type of ADC used determines the settings available to the operator.

Peak ADC Card

The Peak ADC card supports ASD operation. The Cytometer gives the user the option of 2.5, 5, 10, or 15 microseconds of ASD.

Note: Full Pulse Width (FPW) is not an option with this card. See Figure 2.13-5.

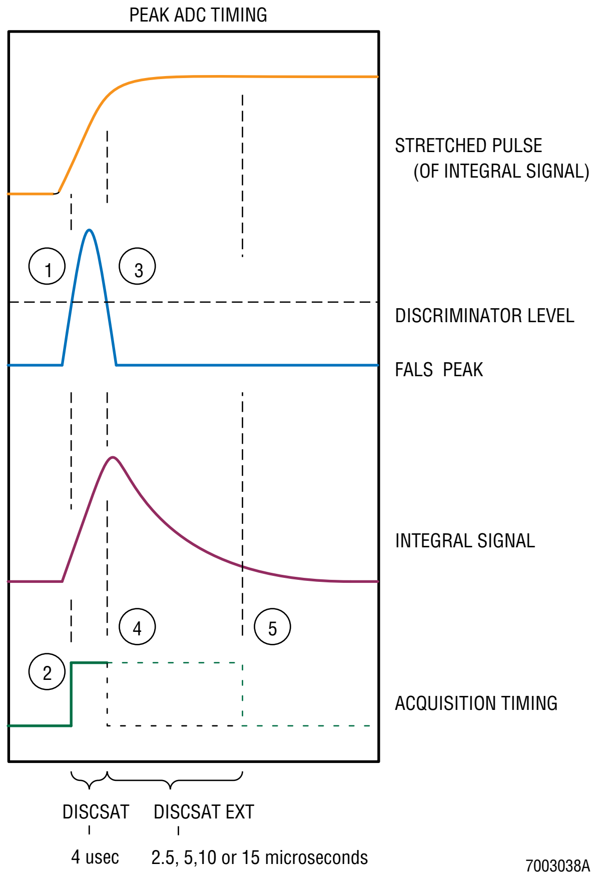
Figure 2.13-5 ASD Settings Diagram



The following chart describes the numbers in Figure 2.13-6.

Number	Description
1	The discriminator level for one Peak Sense and Hold (PSH) channel is exceeded. This is called the trigger channel.
2	All active PSH channels begin capturing data (start of DISCSAT) by allowing their hold capacitors to charge to the input voltage level.
3	The trigger channel's pulse falls below the discriminator level; the DISCSAT period ends. The discriminator extension (DISCSAT EXT) period begins.
4	The DISCSAT EXT period allows slower signals (integral and log) time to reach their maximum values.
5	The DISCSAT EXT ends. The card disables the inputs to the PSH channels; the cards retain the voltage that their respective hold capacitors charged to, until they can be measured by the analog to digital conversion circuit and reset.

Figure 2.13-6 Peak ADC PSH Card Timing



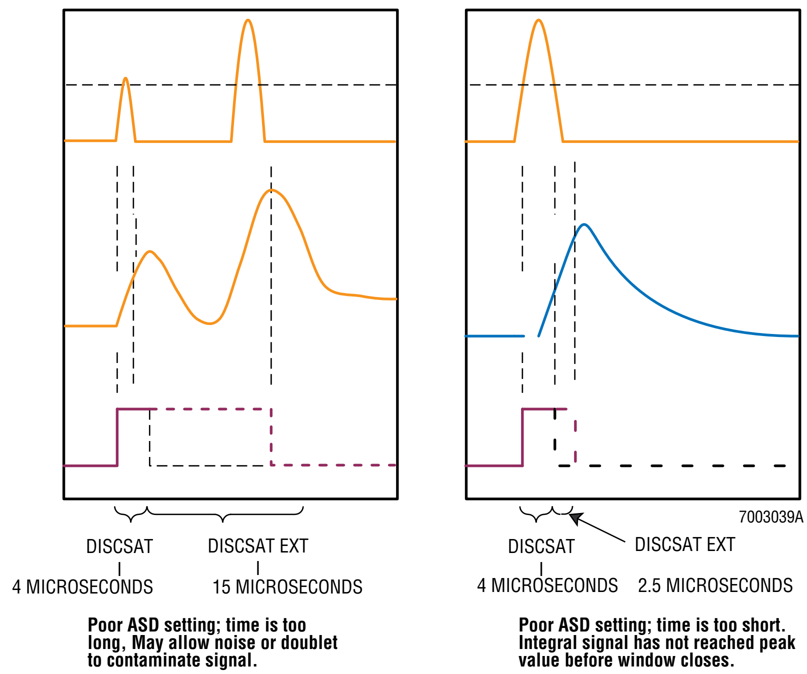
There are several ways to determine the best ASD. You can calculate the time based on the following:

- Laser beam spot size
- Sheath flow velocity
- Average cell size.

An easier method is to observe the pulses on the system's oscilloscope display and to estimate the time needed. It is important that you allow for the slowest rising pulse (integral and log).

With either method, fine-tune the setting by acquiring histograms of the desired parameters. Then, begin with the highest ASD, reduce the time until you notice a shift in the population's mean channel. At that point, set the ASD to the next higher (longer) setting. See Figure 2.13-7 for ASD settings.

Figure 2.13-7 ASD Settings Diagram



ADC and PSH Control Card

The ADC and PSH Control card does not support true ASD operation. During bootup, the presence of this card is flagged by the message *DISCSAT EXTENSION NOT PRESENT*. The Cytometer's OPTIONS screen offers the following choices:

- FPW - Full Pulse Width. The acquisition window starts and stops at the discriminator crossings of the trigger channel. This is the default setting and can be used in most cases if the trigger channel is an integral pulse.

  If a peak signal is used as the discriminator channel, use one of the other two settings.

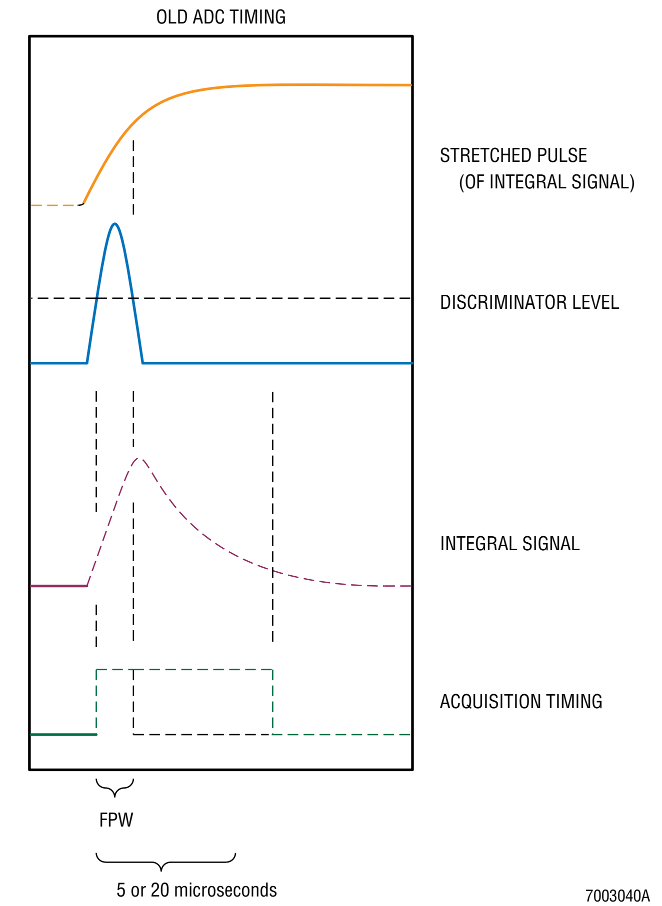
  Note: The minimum acquisition window length is 5 microseconds even if the triggering pulse is shorter than 5 microseconds.

- 5 microseconds. The acquisition window begins at the first discriminator crossing and remains open for 5 microseconds after that time. This setting is appropriate for sense-in-air flow cell (high bandwidth) operation.

- 20 microseconds. The acquisition window begins at the first discriminator crossing and remains open for 20 microseconds after that time. This setting is appropriate for sort sense flow cell operation.

Figure 2.13-8 shows the timing.

Figure 2.13-8 Old ADC Timing Diagram



Lister Out Card

The Lister Out card (Figure 2.13-9):

- Reads the converted channel number values as they are presented on the Data Path bus.

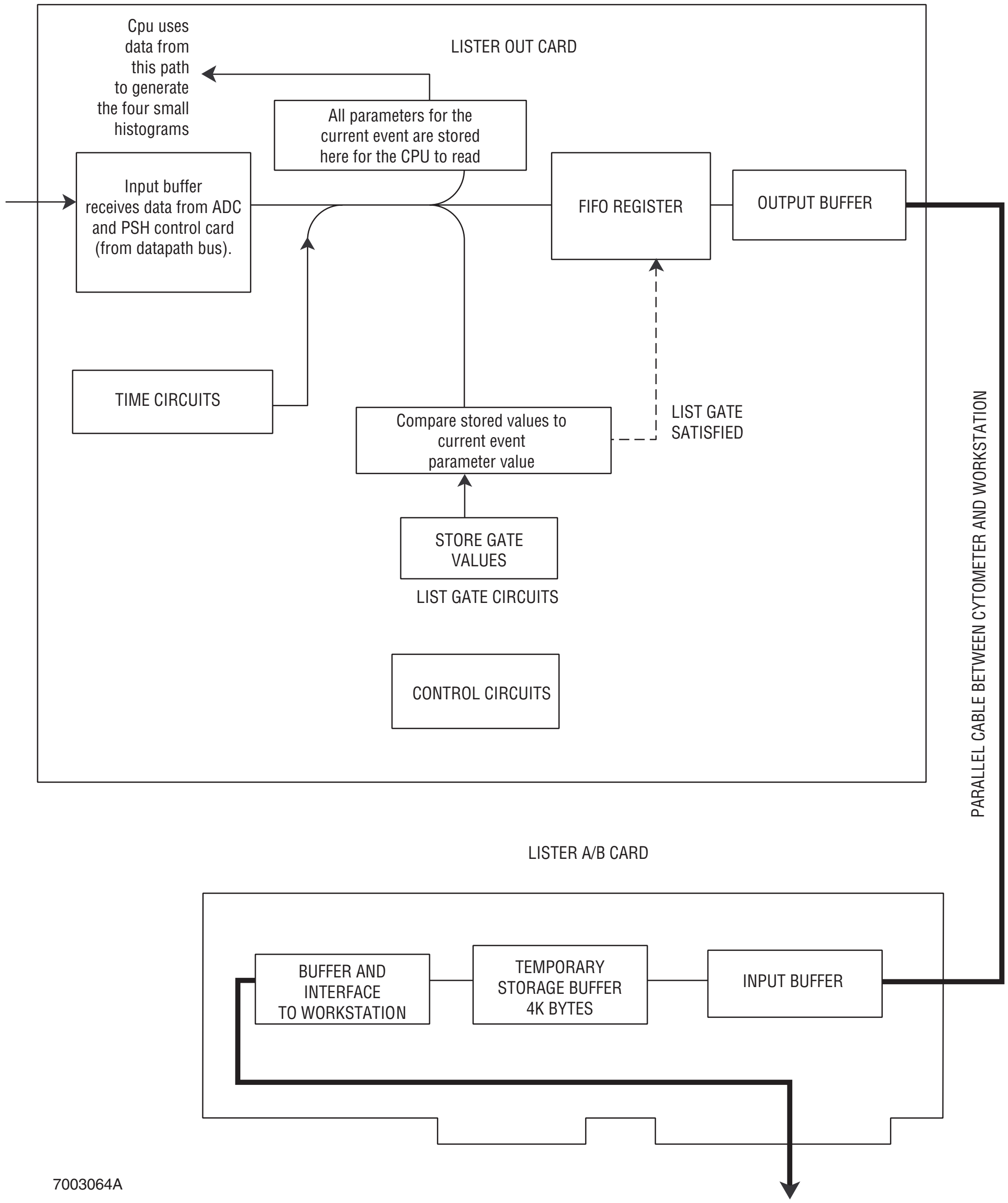
  If time is selected as a parameter, then a time stamp is appended to each group of values. The information is forwarded to the Lister card in the Workstation via the parallel connecting cable. The Cytometer CPU samples the converted values (over the backplane) to generate the mini-histograms on the Cytometer screen.

- Provides the List Gate function. Refer to Heading 5.8 in the Operator's Guide for more information.

  The necessary hardware to store the list gate window and to compare the appropriate datapath parameters resides on this card.

  Note: If it is possible to obtain acquisition on the Cytometer screen and not on the Workstation monitor, then the problem is clearly isolated to one of the Lister cards or to the parallel cable or Lister A/B in computer.

Figure 2.13-9 Block Diagram, Lister Cards



Regarding Figure 2.13-9:

- Time circuits start counting elapsed time when acquisition starts. The time for the current event can be appended to the data stream as the last parameter if time is selected as a parameter.
- Control Circuits determine if data leaves the Lister card (Acquisition ON/OFF). The control circuits also select parameters to be tested for the list parameters to be tested for the list gates, stores the gate region values and determines how many parameters to send for each event.

- FIFO Register stores all the parameter values for the current event. Values are only readout if the list gate is satisfied.
- Output Buffer sends the data to the Workstation.
- Input Buffer receives the data from the Cytometer.

Lister A/B Card

This card resides in the Workstation computer.

The Lister A/B card:

- Receives the acquired data from the Cytometer
- Provides a buffer and interface to the Workstation computer

PART B: OPTIONS AND UPGRADES INSTALLATION

ILLUSTRATIONS

TABLES

PART A: INSTRUMENT INSTALLATION

WARNING Risk of personal injury. Installation of an Elite system by anyone who has not successfully completed a Beckman Coulter authorized training course is prohibited. Avoid personal injury by obtaining the necessary training to complete these installation procedures.

3.1 PREINSTALLATION CHECK

Before installing the instrument, verify the following conditions:

- Education Center training
- Cartons received
- Power requirements
- Water
- Drainage
- Environment
- Ventilation
- Space and accessibility
- Labels
- Supplies

Education Center Training

Verify that at least one person from the customer laboratory is already trained or is assigned to a class date within two weeks of installation.

Cartons Received

1. Compare the cartons received against the customer's order, and notify shipping if there is a discrepancy.
2. Inspect the cartons for damage. If any damage exists, confirm that a claim was filed with the carrier.

Power Requirements

Power requirements vary for each country. It is important that the correct electrical input is available prior to installation.

WARNING Risk of personal injury. Ensure that there is a main power disconnect switch in the same room as the lasers to avoid personal injury.

1. Verify that there are sufficient power outlets available. See Table 3.1-1.
2. Verify that there is an appropriate power outlet for any accessories, including printers and optional disk drives.

Table 3.1-1 Power Requirements

Country	System Power	Laser Power	Omnichrome 56 HeCD Laser*	Coherent 305 Water Cooled Laser*†
USA and other applicable countries	115 Vac, 50/60 Hz at 20 A	115 Vac, 50/60 Hz at 20 A	115/220 Vac, 50/60 Hz, Power Consumption <300 W	208 Vac ±10%, 3-phase with ground, no neutral 50/60 Hz. Recommended capacity: 60 A per phase. Current consumed: 50 A per phase
Europe and other applicable countries	220 Vac, 20/60 Hz at 10 A	220 Vac, 50/60 Hz at 10 A		

* Optional laser

† The electrical service for water cooled lasers may be either WYE or Delta connected with the fourth wire to the building's ground. WYE is recommended because it is more tolerant to line voltage imbalances from each phase to ground.

Water

A system with an optional Coherent Model 305 Laser requires a source of water that meets the specifications in Table 3.1-2.

Table 3.1-2 Water Specifications for Coherent 305 Laser

Description	**Specification**
Flow rate per minute	Minimum 8.5 liters (2.2 gallons) Maximum 11.6 liters (3.0 gallons), recommended
Static pressure **Note:** Static pressure is the inlet pressure measured under conditions of zero flow.	Maximum 620 kPa (90 psi)
System CV	0.47
Pressure Delta **Note:** Pressure Delta is the pressure differential between inlet and drain.	152-276 kPa (22 - 40 psi)
Inlet temperature	10 - 32°C (50 - 90°F)
Temperature stability	±1°C (1.8°F)
Resistivity	5.0 KΩ-cm
pH	6 - 8
Hardness	<100 mg/l (5.9 grains/gallon) or 100 parts/million of calcium
Particulate size	<200 μ diameter (Coherent supplies a 60 μ water filter with the laser.)
Total heat load	20 kW at 230 Vac, 18 kW at 208 Vac

The specifications in Table 3.1-2 include the two 7.6 m (25 ft.) of 16 mm (5/8 in.) i.d. hose supplied with each laser. However, these specifications do not include a 60 µ water filter which, when new, introduces a 5 kPa (0.75 psi) pressure delta at 22.7 liters (6 gallons) per minute or a 2 kPa (0.30 psi) pressure delta at 8.3 liters (2.2 gallons) per minute.

Drainage

Drainage must be able to accommodate the water from lasers, if any.

Environment

The room where the Elite is to be installed must meet the specifications in Table 3.1-3.

Table 3.1-3 Environmental Requirements

Description	Specification
Humidity	0 - 90%, non-condensing
Heat dissipation into room	2,300 W (7,850 Btu/hr) with one air cooled Argon laser 2,400 W (8,191 Btu/hr) with one air cooled Argon laser and one water cooled laser 2,400 W (8,191 Btu/hr) with one air cooled Argon laser and one Omnichrome Model 56 HeCd laser

CAUTION Risk of instrument damage. For systems with water cooled lasers, the cooling water temperature must not fall below the ambient dew point, otherwise, condensation forms inside the power supply and may cause catastrophic damage. In some situations, it may be necessary to provide humidity control for the laser environment.

Description	Specification
Ambient temperature	18° - 29°C (64° - 85°F)
Stability/rate of change	Temperature fluctuations within the ambient temperature range can affect sorting performance. For optimal sorting performance, Beckman Coulter recommends the room temperature not fluctuate more than 1°C (3.5°F) in an hour during sorting.

Space and Accessibility

The Elite standard configuration consists of:

- One 15 mW air-cooled Argon laser, and
- One 10 mW HeNe laser.

The configuration for an Elite with optional laser(s) is the same as the standard configuration plus:

- HeCd laser, and/or
- Water-cooled laser;

See Table 3.1-4 for the unit size and necessary operating clearances for each configuration.

Table 3.1-4 Required Operating Clearance

Specifications	Instrument Configuration: Elite Standard Configuration*	Instrument Configuration: Elite with Optional Laser(s)†
Width	142 cm (56 in.)	173 cm (68 in.)
Additional clearance on right	46 cm (18 in.)	46 cm (18 in.)
Additional clearance on left	5 cm (2 in.)	15 cm (6 in.)
Total clearance needed	192 cm (76 in.)	234 cm (92 in.)
Length	170 cm (67 in.)	170 cm (67 in.)
Additional clearance in rear	46 cm (18 in.)	46 cm (18 in.)
Total clearance needed	216 cm (85 in.)	216 cm (85 in.)
Height	130 cm (51 in.)	130 cm (51 in.)
Additional clearance on top	46 cm (18 in.)	46 cm (18 in.)
Total clearance needed	176 cm (69 in.)	176 cm (69 in.)

**Consists of one 15mW air-cooled Argon laser and one 10 MW HeNe Laser*
†Same as standard configuration plus an additional HeCd laser and/or water-cooled laser.

Optional Printer

The instrument's table can accommodate an optional Printer. However, the user may prefer to use a separate Printer stand to free up the work space.

Labels

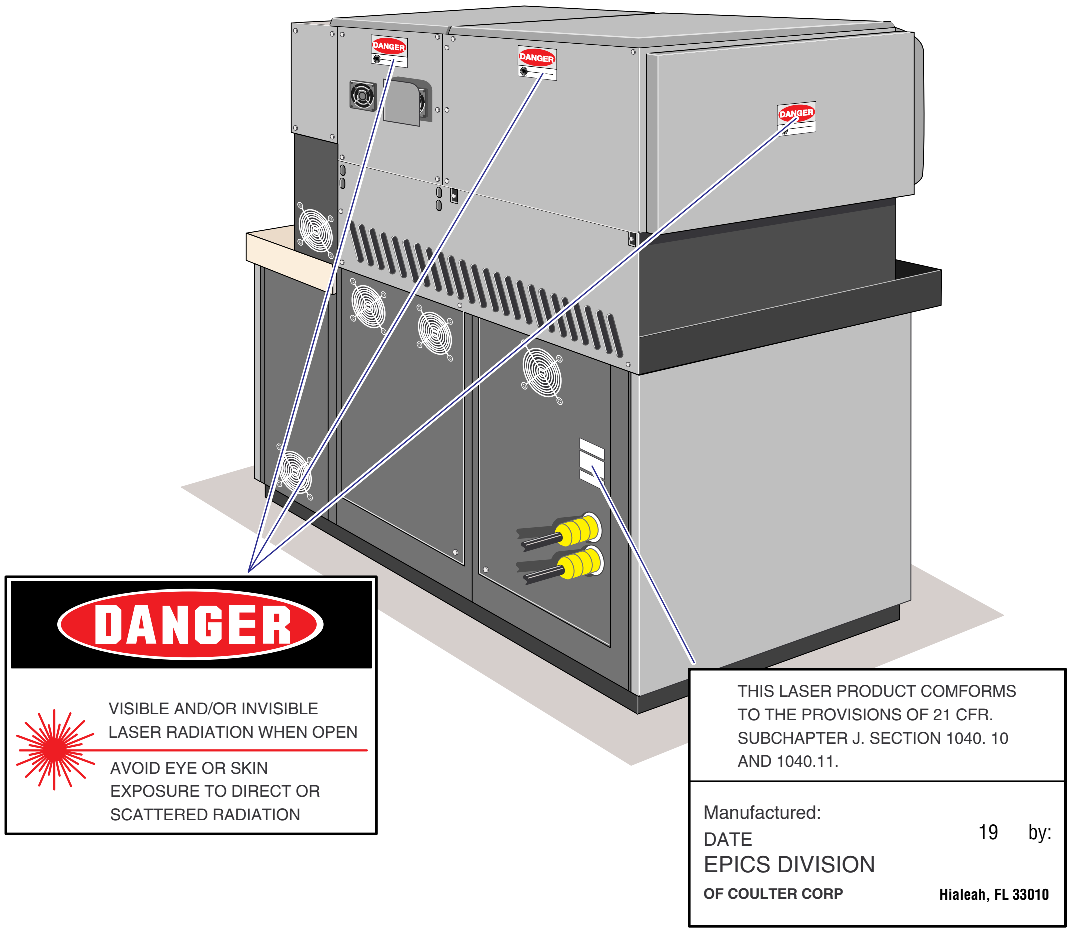
Verify that all labels are in place on the instrument. See Figure 3.1-1.

Supplies

Verify that the necessary supplies are available. The supplies include:

- IsoFlow™ sheath fluid, 3 liters
- Fluorospheres
- Deionized water, 3 liters

Figure 3.1-1 Warning Labels on Instrument



7003094A

3.2 INITIAL SETUP

Unloading and Moving the Instrument

The instrument arrives at the customer site with the Cytometer packed in a palletized box. The remainder of the instrument arrives in boxes that are not palletized.

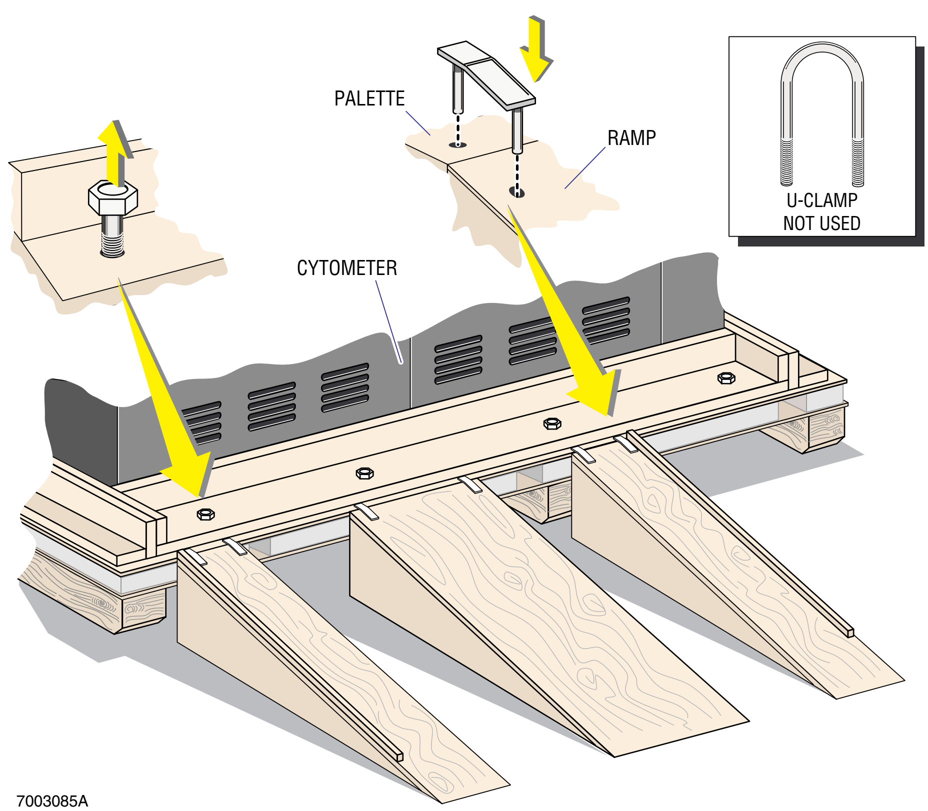
WARNING Risk of personal injury. Do not attempt to unload the main assembly without using a fork lift or a pallet carrier. Failure to use a fork lift or pallet carrier can result in serious personal injury.

1. Ensure that the Cytometer is removed from the delivery truck with a fork lift or pallet carrier.
2. Inspect all boxes for damage. Notify shipping of any external damage.
3. Remove the banding and cardboard cover from the Cytometer.
4. Unbolt the wooden bracket that secures the Cytometer to the pallet as shown in Figure 3.2-1.

CAUTION Risk of instrument damage. Be sure to properly secure the special ramps with the clips provided. Do not use the U-shaped clips. Use the flat clips.

5. Place the ramps in front of the Cytometer's pallet. See Figure 3.2-1.

Figure 3.2-1 Unloading the Cytometer



6. Secure the ramps to the pallet with the flat drop-in clips as shown in Figure 3.2-1.
7. Verify that the ramps are properly aligned with the pallet.
8. Roll the Cytometer on its built-in casters down the ramps and to the desired installation location.

   Note: The Cytometer requires a moving clearance of 143 cm (56 in.) by 77 cm (30 in.) by 130 cm (51 in.).
9. If the Elite configuration includes an optional water cooled laser, Innova 300, instruct the customer to store the crates in case the laser has to be sent back to the factory for service.

   Note: The specifications of the packed laser are the following:

   - Power Supply, crated:
     - Length of 91 cm (36in.)
     - Width of 74 cm (29 in.)
     - Height of 48 cm (19 in.)
     - Weight of 28 kg (62 lb.).
   - Laser Head, crated:
     - Length of 201 cm (79 in.)
     - Width of 53 cm (21 in.)
     - Height of 48 cm (19 in.)
     - Weight of 39 kg (86 lb.)

Dual CRT Installation

If the Elite system arrives without the dual CRT assembly attached, perform the following additional steps:

- After you move the Cytometer to the installation location, and
- Before you attach the computer pedestal to the table top.

1. Verify that the system is disconnected from the main power source.
2. Remove the inner access panel under the PMTs.

ATTENTION: It is a good idea to have someone assist you with sliding the dual CRT assembly onto its post.

3. Slide the dual CRT assembly onto its post as shown in Figure 3.2-2.

IMPORTANT Risk of misleading results. Cables that are caught or pinched can cause misleading results. Ensure that the cables are not caught or pinched.

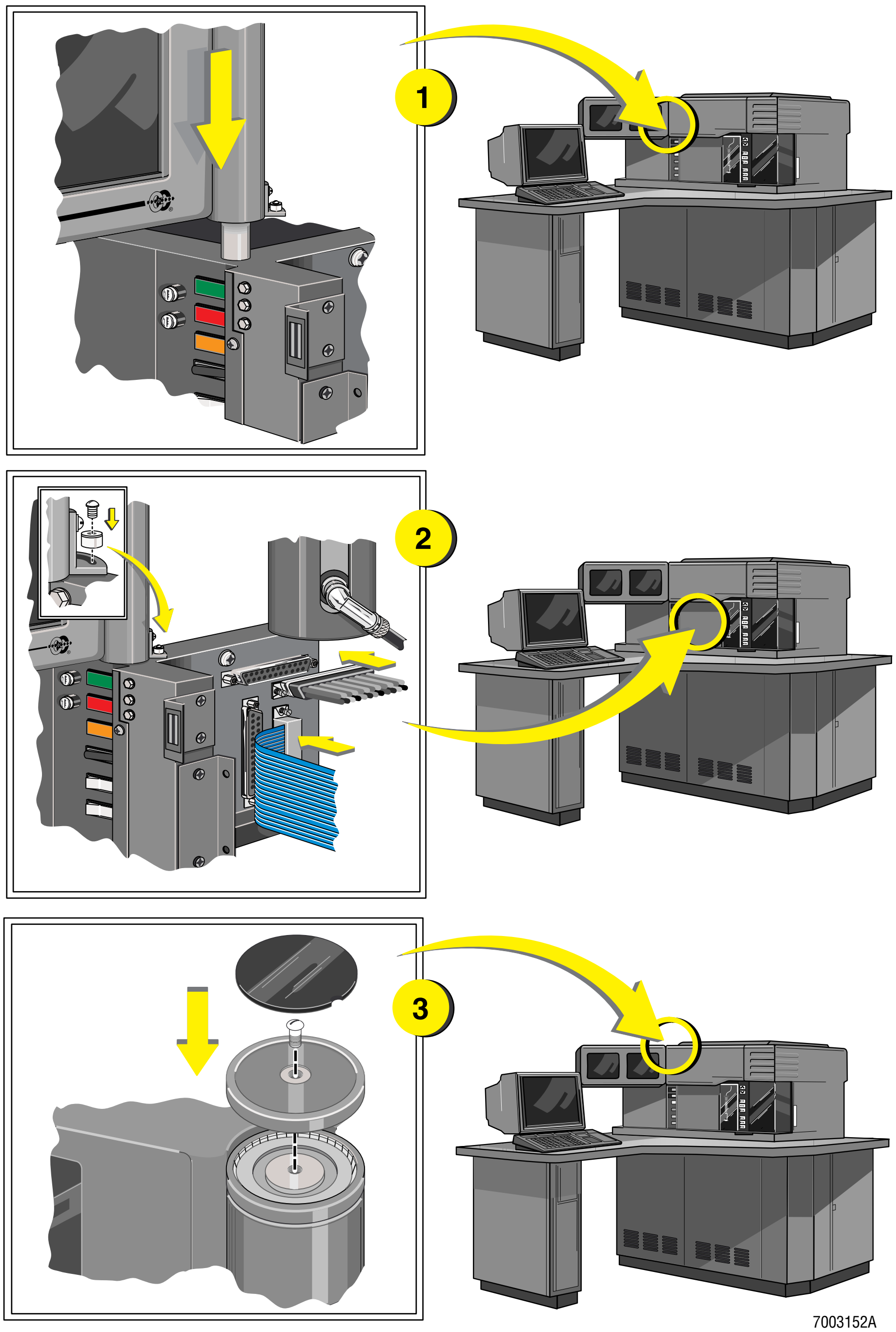
4. Ensure that the connecting cables pass through the cover's slot into the instrument.

   Note: If the upper bearing was pushed out, reinstall it before proceeding.
5. Attach the screw and cap to the top of the post as shown in Figure 3.2-2.
6. Install the limit screw and plastic washer at the base of the post as shown.
7. Install the cable connectors as shown. Be careful not to overtighten the mounting screws.

IMPORTANT Risk of misleading results. A pinched sample line or fiber optic cable can cause misleading results. Use care to avoid pinching a sample line or fiber optic cable.

8. Reinstall the inner panel.

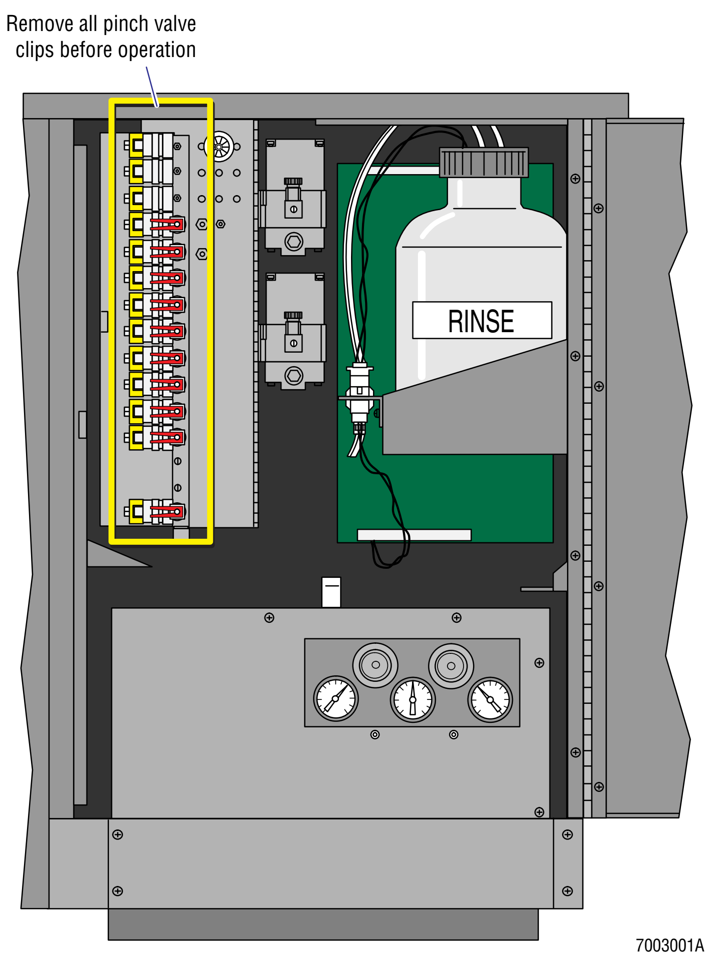
Figure 3.2-2 Dual CRT Installation



Instrument Installation

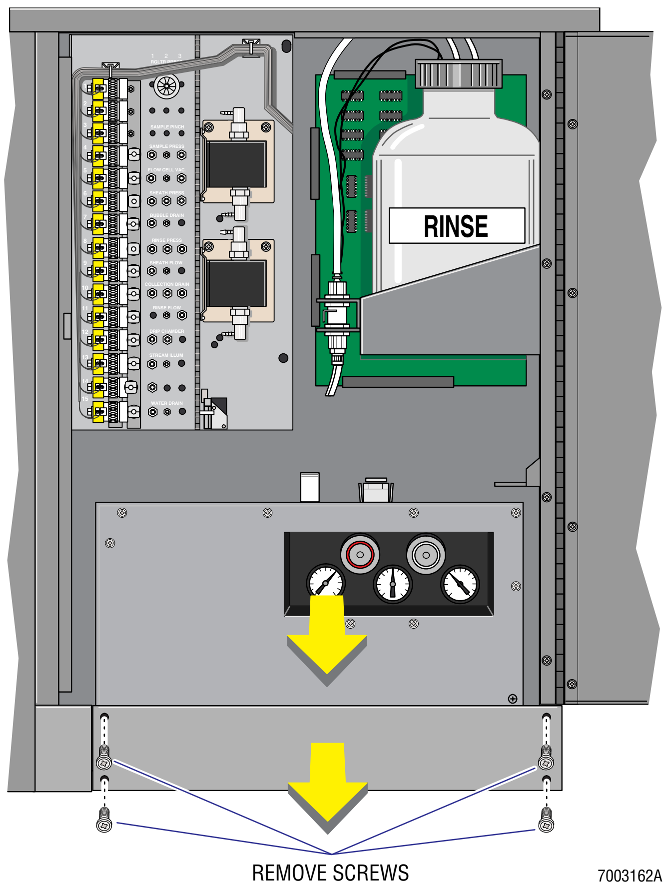
1. Place the computer pedestal in its proper location.
2. Install the table top as follows:
   a. Insert the table top into the brackets on the front of the Cytometer.
   b. Rest the free end of the table top on the computer pedestal.
   c. Secure the table top to the Cytometer with the screws provided.
3. Remove the front cover from the computer pedestal, and slide the computer into the pedestal from the front.
4. Verify that the computer power switch is at the top of the pedestal and is in the ON position.
5. Remove the keys from the back of the computer and put them in the filter box.
6. Place the color monitor, mouse, and keyboard in the correct locations on the table top.
7. Remove the rinse bottle holder (Figure 3.2-3):
   a. Open the pneumatics compartment door.
   b. Loosen the screws that hold the rinse bottle holder in place.
   c. Remove the rinse bottle holder.

Figure 3.2-3 Rinse Bottle Holder Location



8. Position the Compressor module to the floor:
   a. Lift up on the Compressor module handle.
   b. Remove the four screws that hold the Compressor to the brackets on the side of the pneumatics compartment as shown in Figure 3.2-4.
   c. Lower the Compressor to the floor.
   d. Position the Compressor so none of it touches the instrument.

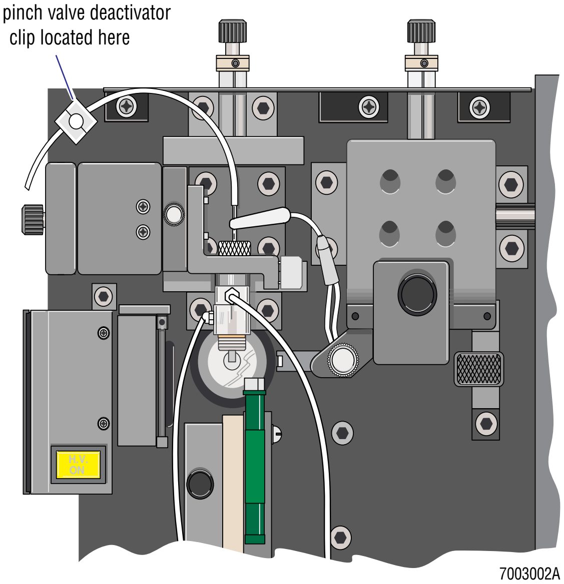
Figure 3.2-4 Screws Securing Compressor to Pneumatics Cabinet



9. Reinstall the rinse bottle holder.
10. Remove the pinch valve deactivator clips.

Note: There is a pinch valve deactivator clip located in the flow cell area as shown in Figure 3.2-5.

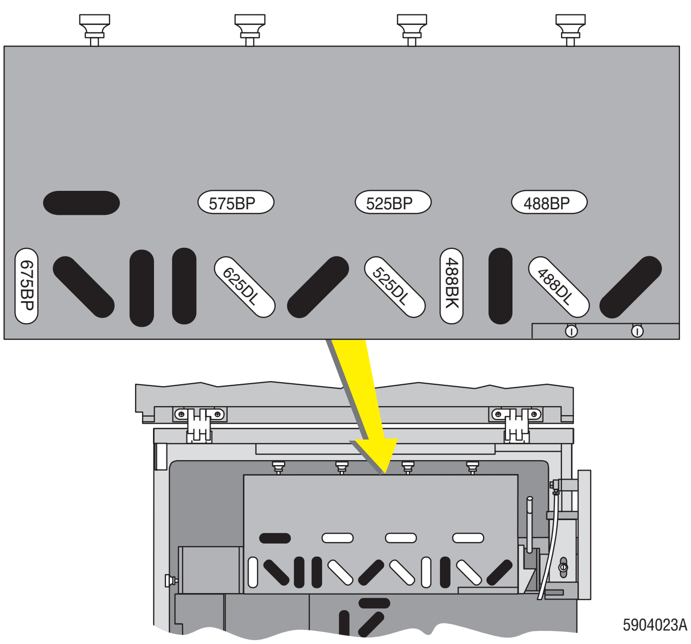
Figure 3.2-5 Pinch Valve Deactivator Clip



11. Install the filters:
    a. Open the filter compartment.
    b. Insert the filters from the filter kit into the appropriate slots. See Figure 3.2-6.

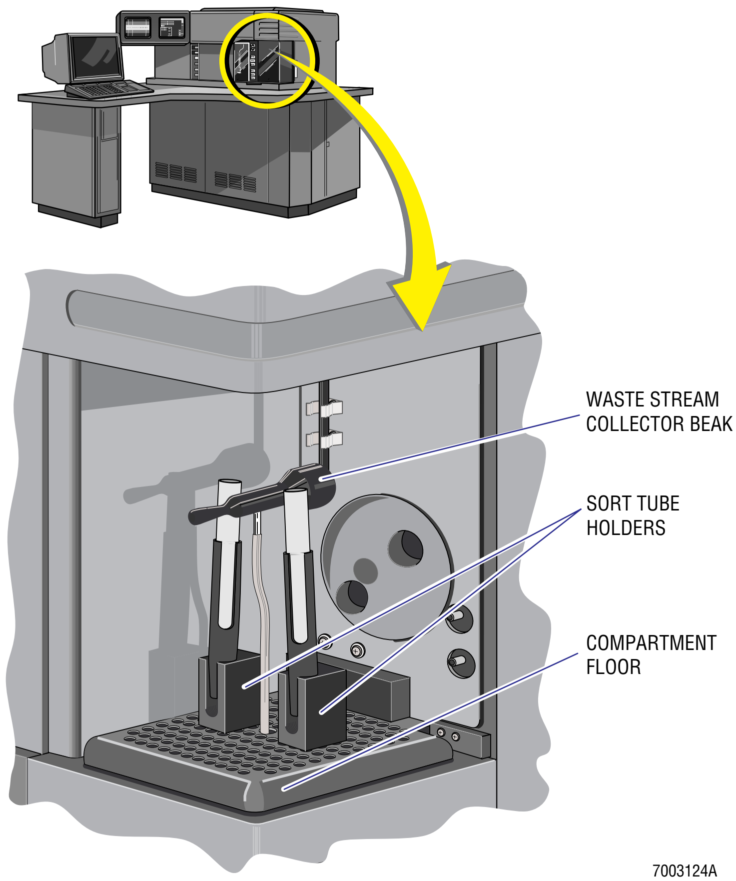
ATTENTION: If installing an Elite Analyzer, skip step 12 and go to Heading 3.3.

Figure 3.2-6 Filter Slots



12. Install the sample collection compartment (Figure 3.2-7) components by installing:
    a. The waste stream collector beak.
    b. The compartment floor.
    c. The sort tube holders.

Figure 3.2-7 Sample Collection Compartment

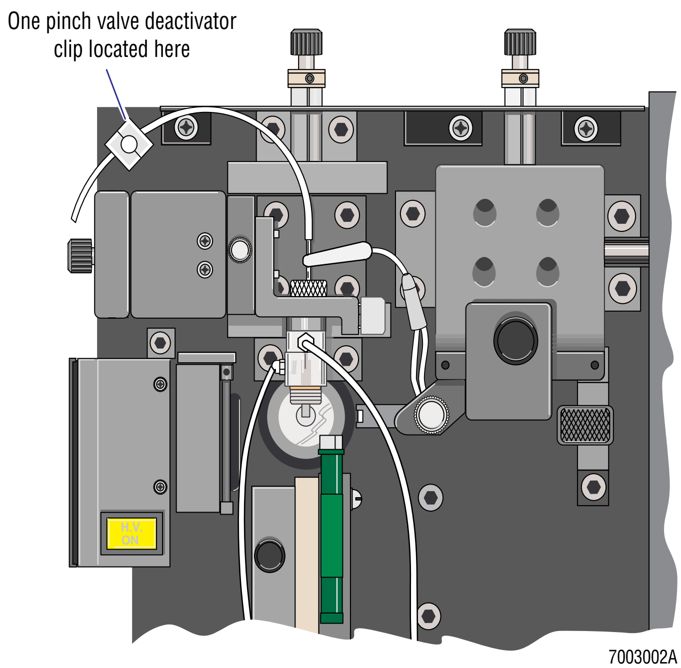


3.3 CYTOMETER CHECK

ATTENTION: Do do remove any of the circuit cards unless instructed to do so.

1. Unbolt and remove the two front panels from the Cytometer.
2. Verify that all circuit cards are properly seated in the rack.
3. Verify that all ribbons and coaxial cables are secure.
4. Open the flow cell compartment door and tilt the cover up by removing the two retaining screws at the rear.
5. If installing the Elite Analyzer, proceed to step 7.
6. Remove the pinch valve deactivator clip from the sample line pinch valve. See Figure 3.3-1.

Figure 3.3-1 Pinch Valve Deactivator Clip



7. Verify that the scatter sensor mask is correctly in place.

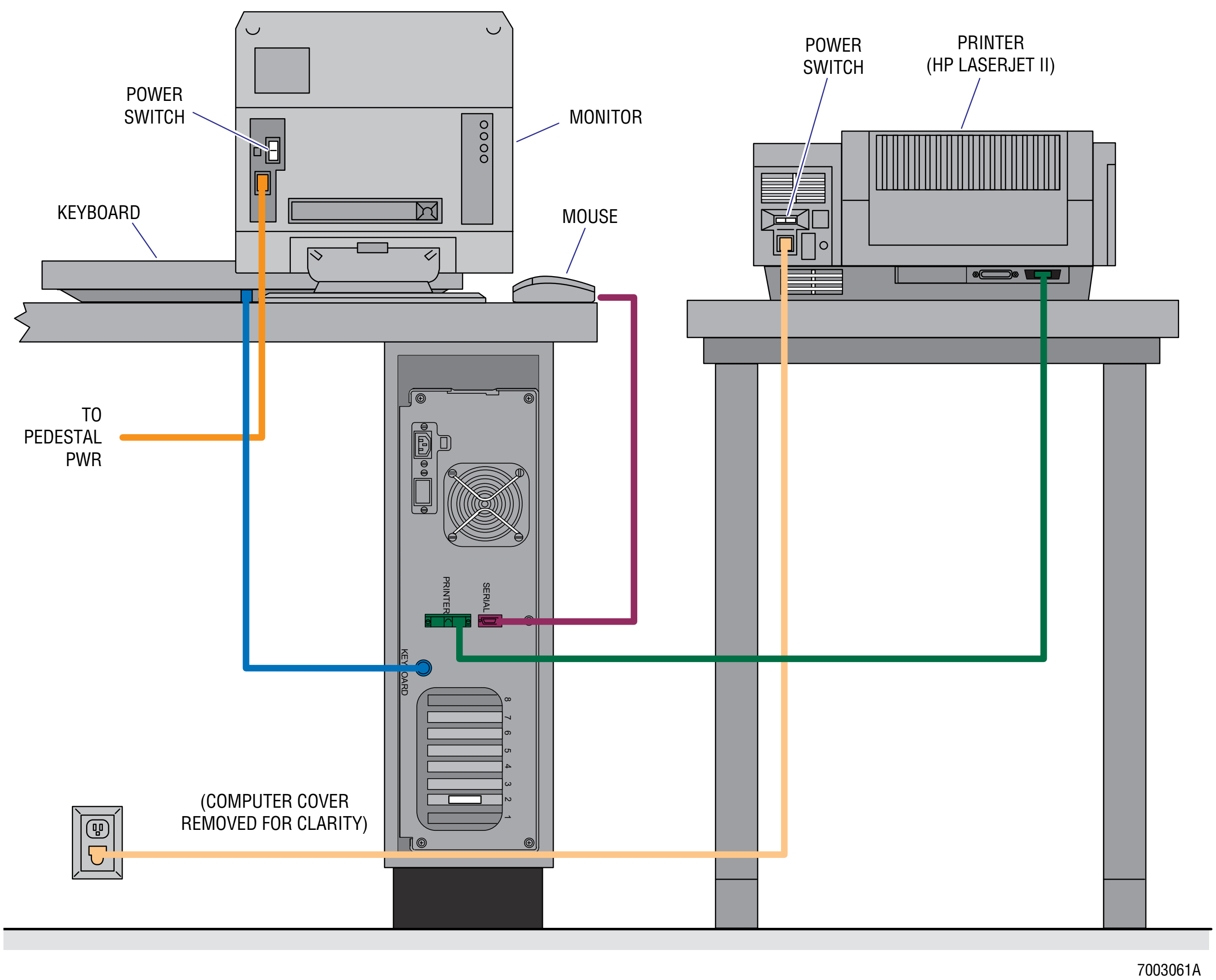
3.4 CONNECTING ASSEMBLIES

Electronic Cable Connections

1. Verify that the two power switches on the Cytometer are in the OFF position.
2. Verify that any optional accessories (printers, optical drives, or lasers) are turned OFF and disconnected from any power source.
3. Inspect all cables to ensure that the pins are straight and not pushed in.
4. Insert the yellow plugs on the two primary power cords into the sockets at the rear of the Cytometer.

   Note: All cables that connect to the computer are routed through the side of the computer pedestal as shown in Figure 3.4-1.

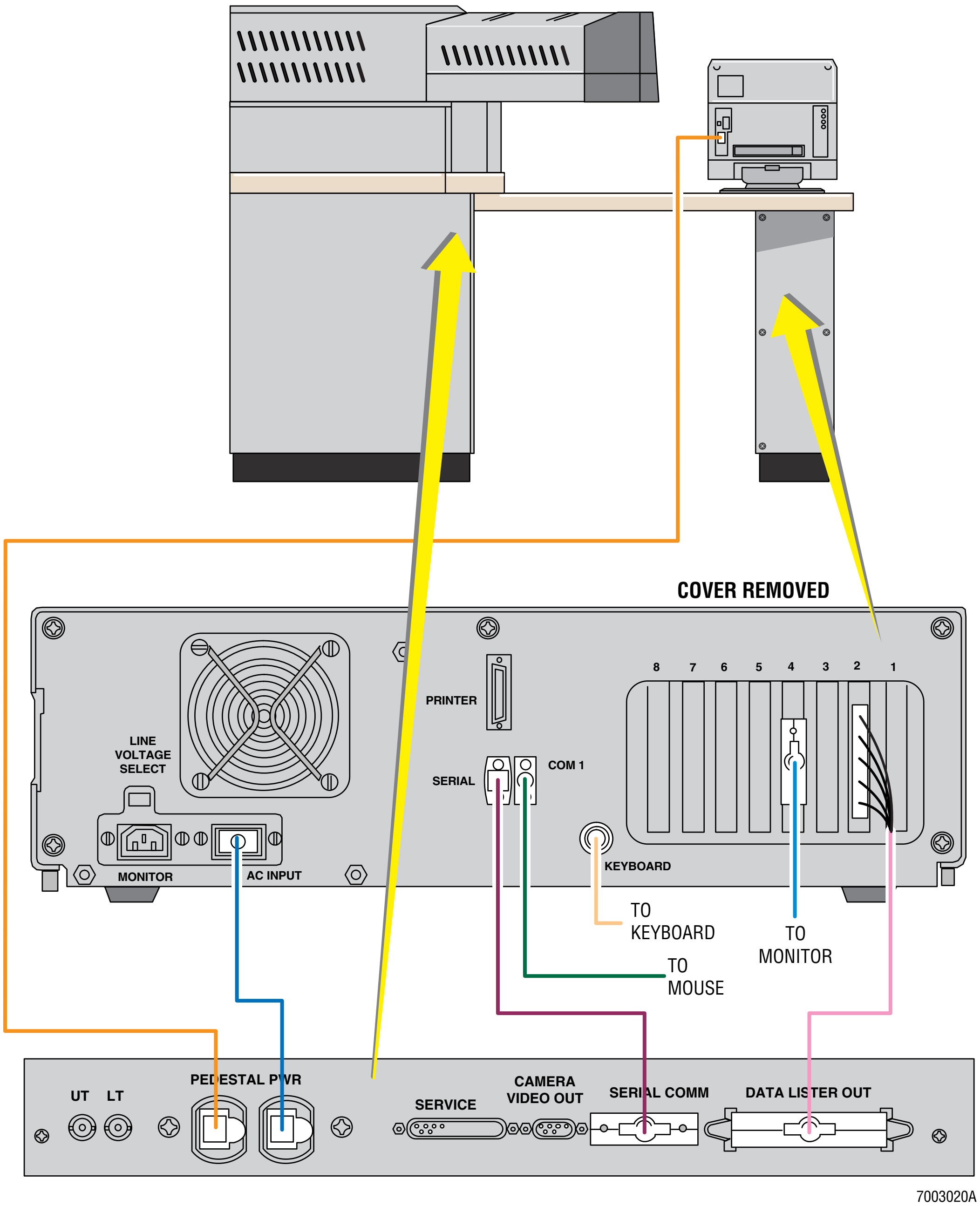
Figure 3.4-1 Electronic Cable Connections



5. Connect the mouse, keyboard and monitor to the computer as shown in Figure 3.4-2.
   a. Connect the mouse to the COM1 port on the computer.
   b. Connect the keyboard to the keyboard jack on the computer.

c. Connect the color monitor to the Video Card connection on the back of the computer.

Figure 3.4-2 Connections to Workstation CPU



6. Connect the parallel data cable:

   a. Connect one end of the cable to the DATA LISTER OUT connector on the Cytometer as shown in Figure 3.4-2.

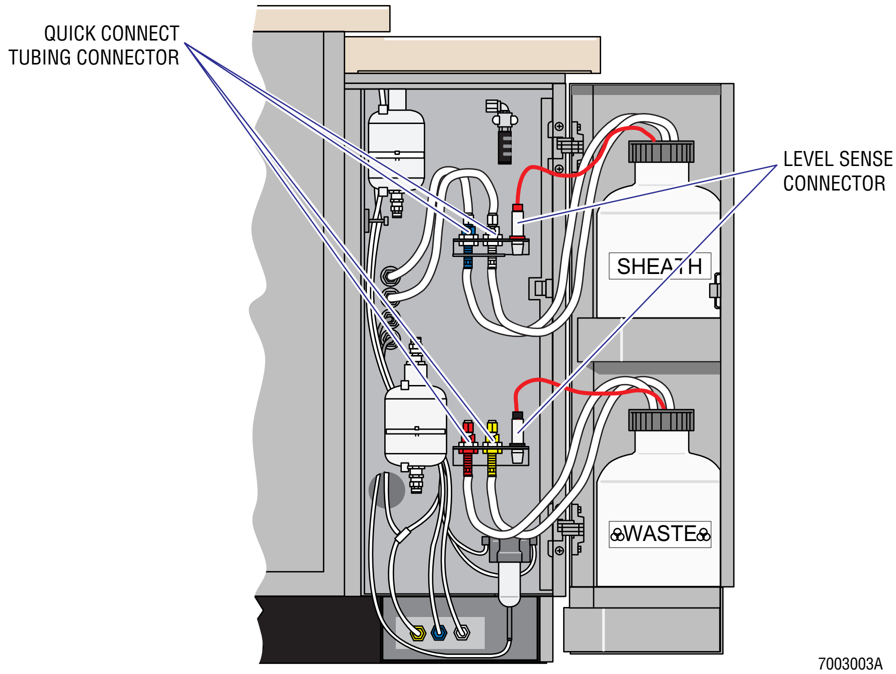
   b. Feed the other end of the cable through the side of the computer pedestal, and connect it to the Lister card connection on the back of the Workstation's CPU.

7. Connect the serial comm cable:
    a. Connect one end of the cable to the Cytometer's SERIAL COMM connector as shown in Figure 3.4-2.
    b. Feed the other end of the cable through the side of the computer pedestal, and connect it to the COM2 port on the Workstation's CPU.
8. Plug the Workstation CPU's power cord into one of the Cytometer's ac receptacles.
9. Plug the color monitor's power cord into the Cytometer's remaining ac receptacle.
10. Carefully press all the cables going to the Cytometer into the wiring channel under the table top.

Tubing Connections

1. Prepare the rinse bottle:
    a. Fill the rinse bottle with deionized water and place the bottle in its holder.
    b. Attach the quick-connect tubing connectors to the appropriate connector. Be sure to match the colors.
    c. Plug the level sense connector into the matching socket.
2. Prepare the sheath bottle:
    a. Fill the sheath bottle with conductive sheath fluid (such as IsoFlow sheath fluid) and place the bottle in its holder. See Figure 3.4-3 for location.
    b. Attach the quick-connect tubing connectors to the appropriate connector. Be sure to match the colors.
    c. Plug the level sense connector into the matching socket.
3. Prepare the waste bottle:
    a. Attach the quick-connect tubing connectors to the appropriate connector. Be sure to match the colors.
    b. Plug the level sense connector into the matching socket.

Figure 3.4-3 Sheath and Waste Bottles



Options Connections

Laser Option

See Heading 3.7, OPTIONAL LASER INSTALLATION for instructions.

Optical Drive Option

The Workstation can be equipped with a number of mass storage drives. For installation information, refer to the information supplied with the drives.

Printer Option

Connect the Printer to the PRINTER port on back of the Workstation's computer.

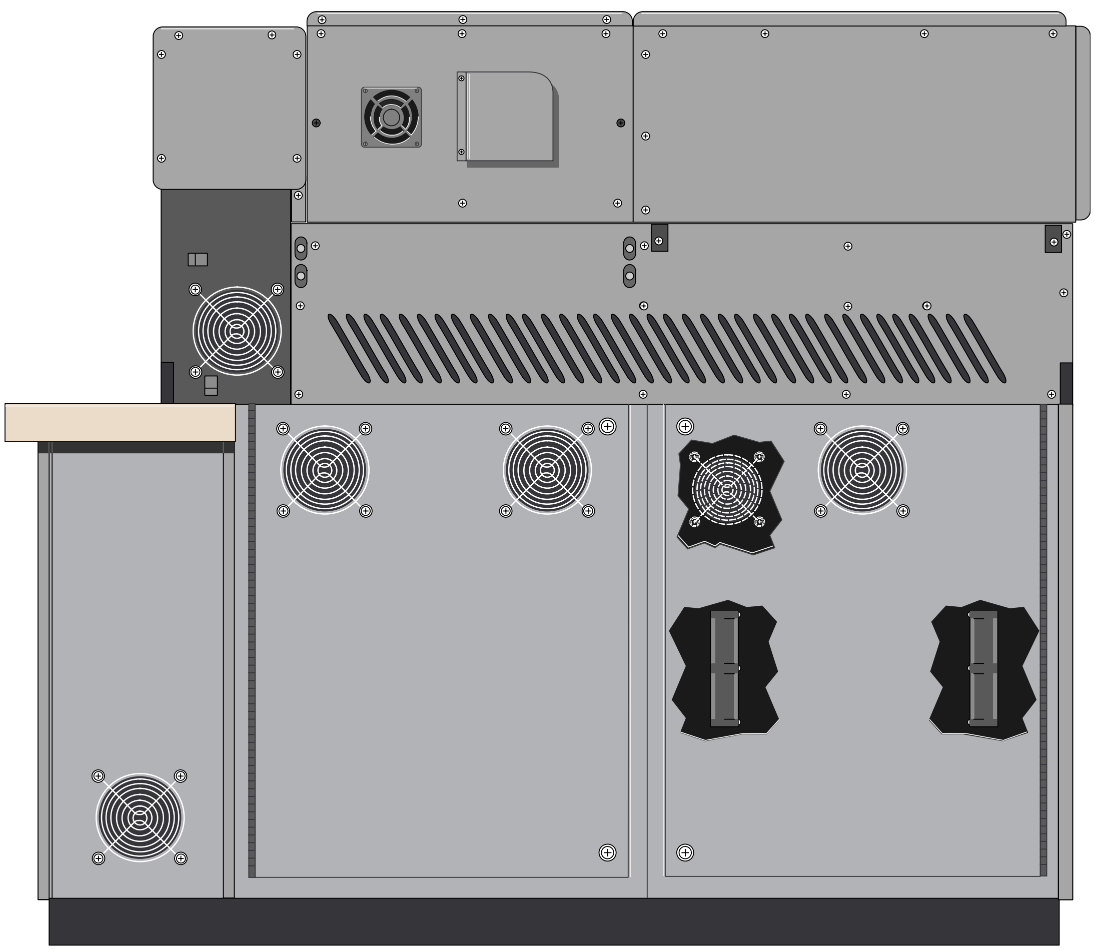
For additional information, refer to the documentation supplied with the Printer.

3.5 TESTING ASSEMBLIES

Cytometer, Control Computer

1. Connect the two Cytometer power plugs to the ac outlets.
2. Turn ON the lower power switch on the Cytometer.
3. Verify that the main control screen (right Cytometer screen) appears within 10 seconds.
4. Verify that all cooling fans (Figure 3.5-1) are operating. With a reference point from the front of the instrument, fan locations are:
    - Two on the right rear door
    - One on the left rear door
    - One above the Gated Amp card cage
    - Two on the Multibus card cage
    - One at the rear of the Cytometer
    - One at the back of the sort collection drawer; the fan is activated by the adjacent switch.

Figure 3.5-1 Fan Locations



7003090A

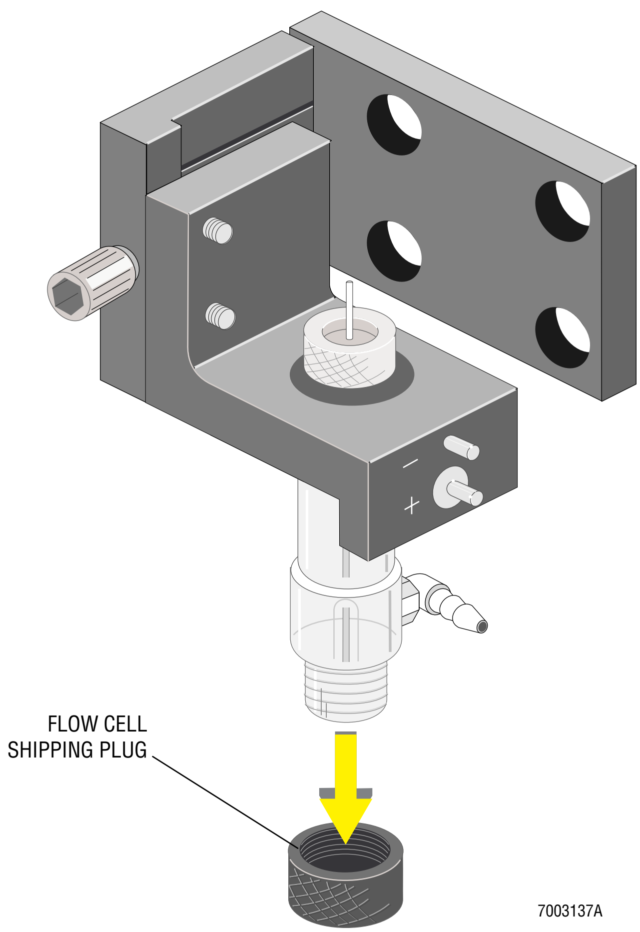
Compressor Module

1. Open the sheath and waste compartment door.
2. Disconnect the center (blue) quick-connect fitting from the lower front of the Cytometer.
3. Verify the system pressure and the compressor pressure is correct:
   a. Open the pneumatics compartment door.
   b. Ensure the system pressure reads 30 psi. Adjust the regulator as necessary.
   c. Ensure the Compressor module's pressure reads 50 psi. Adjust the regulator as necessary.
4. Reconnect the center (blue) quick-connect fitting to the lower front of the Cytometer.

Pneumatics

1. Remove the shipping plug from the flow cell as shown in Figure 3.5-2.

Figure 3.5-2 Flow Cell Shipping Plug Removal



2. Install the Biosense tip.

3. At the Cytometer touch screen, select:
   a. **Power Up Valves**.
   b. **DEBUBBLE** three times.
   c. **Shutdown**.
4. Replace the BioSense tip with the sort sense tip.
5. At the Cytometer touch screen, select **Power Up Valves**.
6. Press **CLEAR** and **DEBUBBLE** as many times as necessary to obtain a stable sheath stream.
7. Examine the flow cell closely for air bubbles. If necessary, press **DEBUBBLE** and **VACUUM** as necessary to remove bubbles.
8. Display the Sheath and Sample Pressures on the Cytometer screen.
9. Verify that both sheath and sample pressures are 12 psi.

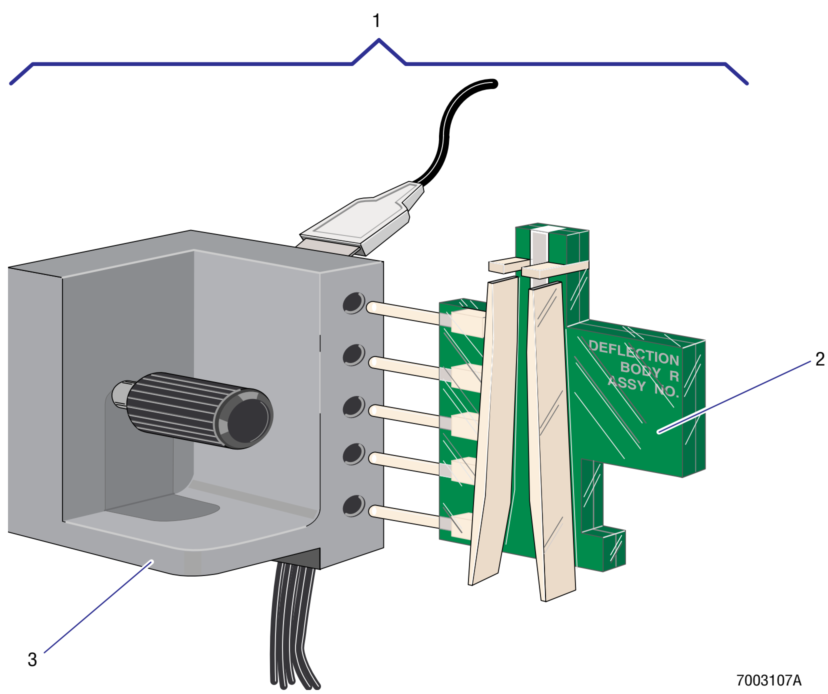
   Note: If the set and read pressures do not agree to ±0.5 psi, do the procedure under Heading 4.5, PNEUMATICS SYSTEM.
10. Verify the sample vial detector is operating correctly.

Camera

ATTENTION: For Elite Analyzer installation, skip this procedure.

1. Install the deflection plate (Figure 3.5-3) and push securely into position.

Figure 3.5-3 Deflection Plate



2. Activate the strobe.
3. Set the left Cytometer screen to the Camera Display mode.
4. Verify that the following criteria are met:

- Stream is centered in the illuminated (strobe) area
- Strobe area is centered on the screen
- Steam and strobe illumination remain centered at all zoom settings
- Stream remains in focus at all zoom settings
- Flow cell alignment image is centered on the screen at all zoom settings when camera is in full up position
- Zoom setting is almost at the mechanical minimum setting, 1.0, of the zoom lens when the zoom is in the full out position
- Zoom or position mechanism does not bind.

Note: If any of these criteria are not met, do Heading 4.1 Camera Adjustment Procedure.

Lasers

WARNING Risk of personal injury. Laser manufacturers of certain accessory lasers may require factory certification of the installer. Do not install any laser unless you have the required training and/or certification.

The alignment procedures depend upon the type of lasers installed and their optical configuration. Refer to the correct laser alignment procedure for details.

Argon Laser

1. Turn ON the Argon laser, if present.
2. Verify operation of the laser cooling fan.
3. Verify light output from the laser, which is approximately 20 second delays.

HeNe Laser

1. Turn on the HeNe laser, if present.
2. Verify operation of the laser cooling fan.
3. Verify light output from the laser.

Optional Lasers

Inspect all optional lasers for correct operation. Refer to the user or service manual supplied with the laser for specifications and procedures.

Computer

1. Turn ON the upper power switch on the front of the Cytometer.
2. Ensure that the Elite menu appears after bootup.
3. Test the system for known software viruses by using the latest available version of virus detection software.

Alignment Check

Do the applicable procedures under Heading 4.1, OPTICAL ALIGNMENT PROCEDURE.

3.6 SYSTEM TESTING

Acquisition Check

1. Create a protocol to acquire fluorospheres as follows:
   - Histogram 1: FALS vs. 90LS, stop at 5,000
   - Histogram 2: FALS
   - Histogram 3: 90LS
   - Histogram 4: Log GFL (PMT2)
   - Histogram 5: GFL
   - Histogram 6: Peak GFL
   - Histogram 7: RFL (PMT3)
   - Histogram 8: PMT4
   - FALS Discriminator: 100, all others OFF
   - Gains: 10
   - HV: 400
2. Set Cytosettings to SEND.
3. Ensure all values are transferred, then select **Acquisition ▸▸ Start**.
4. Ensure that CVs obtained on GFL are comparable to the specifications of the fluorospheres used.
5. Ensure via the pulse display that the integral, peak, and log signals are generated for all PMTs.
6. Vary discriminator setting to ensure correct operation.
7. Save eight histograms and two Listmode files.
8. Use an appropriate protocol and analyze Cyto-Trol™ control cells or samples stained for T4, T8, and B4.

   Note: Be sure to include a PE vs. FITC gated on FALS vs. 90LS histogram.

Analysis Check

1. At the Workstation Listmode screen, verify operation of the Listmode playback.
2. At the Workstation Multigraph screen, verify operation of stored histogram analysis.

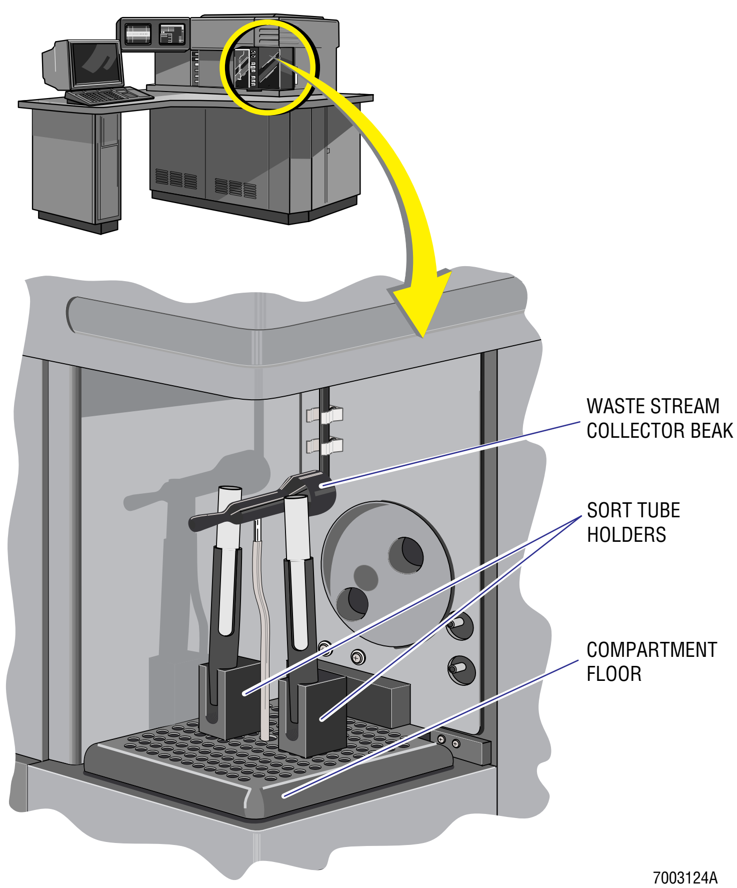
Sort Check

Note: This procedure does not apply to Elite Analyzer systems.

1. If the instrument has been shut down:
   a. Perform the Startup procedure.
   b. Check for proper alignment and flow and perform the cleaning and adjustment procedures as needed.
2. Clean the deflection plate if saline is evident.
3. Plug in the deflection plate.

4. Place the top of the strobe between the bottom of the beam shaping lens and the bottom of the lens holder.
5. Center the stream within the sort plate's opening by adjusting the knob on the deflection body holder to center the ground plate opening around the stream.
   - If the stream is still too close to one side, the other Z-axis of the flow cell stage is not adjusted correctly. Ensure that this is set correctly through proper alignment before continuing.
   - Any movement of the Z-axis can affect the rest of the optical alignment.
   - After a new flow cell, flow body, or bimorph stage is replaced, you should only need to adjust the knob once.
6. Set the Sort settings. Refer to Drive Frequency, Phase Adjustment, and Delay Calculation in this section for details.
7. Sort a mixture of different brightness beads 50,000 right and left. Refer to the settings obtained in step 6. Refer to Figure 3.6-1 for placement of tubes for sorting.
   - For non-ESP systems, sort at 1,500 events/sec data rate, with Abort ON.
   - For ESP systems, sort at 5,000 event/sec, with Abort ON/Complete Abort.

Figure 3.6-1 Tube Placement for Sorting



8. Reanalyze the sorted beads to determine purity:
   - >95% for non-ESP systems
   - >99% for ESP systems.

Drive Frequency

1. Set the **Drive Frequency** to 32 kHz.
2. Set the **Crystal Drive** to 75%.
3. View the droplets above the ground plate, and scan through the frequency settings using the tenth decimal adjustment.

   Note: It is recommended that you start at 32 kHz and scan down.
4. When the highest breakoff is achieved and the droplets appear very focused and sharp, stop scanning.

   Note: Highest breakoff means closest to the flow cell.
5. Set a cursor tangent to the bottom of the last attached droplet.

   Note: If the last attached droplet is not yet visible above the top of the ground plate, increase the crystal drive from 75% to 85%. If the droplet is still not visible, move the deflection body slightly down.
6. Turn OFF the **High Voltage**.
7. Press **DEBUBBLE** twice, and verify that the breakoff returns to the same place.

   If the breakoff does not return to the same place, the flow cell has a bubble or is partially clogged. Remedy this situation before continuing.

   Note: You may have to remove or replace the flow cell.
8. Move the camera all the way down stream and look at the droplets. The droplets should be clear, sharp, and fairly round in shape; there should be no satellite droplets.
   - If there are satellite droplets, then proceed to step 9.
   - If there are no satellite droplets, slightly adjust the frequency via the decimal setting to improve the shape and clarity of the droplets.
9. Move the camera back upstream and ensure that the last attached droplet position has not changed by more than three droplets.
   - You may have to adjust the sort deflection body in order to view the last attached drop.
   - It is helpful if the last attached droplet is visible just above the ground plate, so that you can easily monitor it.

Phase Adjustment

1. Set the **Sort Counters** to OFF.
2. Select:

   a. **Sort Test**.

   b. **Left Sort** and **Right Sort**.

   c. **High Voltage**.

3. Set the **Sort Droplet** to 3 and press **START** to activate the sort counters. Sort counters should be running.

IMPORTANT Risk of incorrect results. If the side streams hit the sort plates, incorrect results may occur. Do not allow the side streams to hit the sort plates.

4. Increase the deflection by increasing the Deflection amplitude to bring out the side streams.
5. Find the best side streams by using the phase adjustment, which is the decimal portion of the Delay setting. You should get at least seven of the phase settings to provide good, clean side streams. If not, repeat step 4.

   Note: The problem may be an incorrect Drive setting. Optimal side streams are evident when the filament between the two drops is very thin and remains connected to the drops.
6. Find the worst phase setting and subtract 0.5. Set the phase to that number to provide the best side streams.
7. Deselect **Sort Test** to turn off.
8. Load the sample protocol created in step 1 of Acquisition Check.
9. Set the **Sort Settings** to **Receive**.
10. Send the **Cyto-Settings**.
11. Change the Sort Settings to Send to send your sample sort gates.

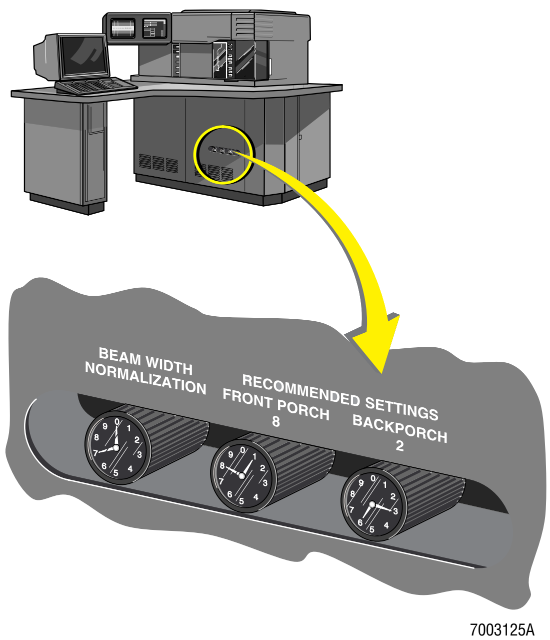
IMPORTANT Risk of compromising sort stability if you increase the sample pressure more than 0.20 psi above optimal setting for running beads. Avoid compromising sort stability; do not increase the sample pressure more than 0.20 psi above optimal setting for running beads.

12. Run the sample by either of the following methods:
    - Run the actual sample at the data rate desired for sorting. Note the sample pressure required to achieve the desired sort rate and use this same sample pressure when doing the sort matrix, which means checking the Delay setting.

    OR
    - Run fluorospheres at the same concentration as the sample to be sorted.
13. Examine the side streams while the sample is running.
14. Use a 3-drop sort to ensure that the side streams are as clean as possible.
    a. To make the streams cleaner, you may need to adjust the Front Porch and Back Porch adjustments.
       1) For ESP systems, the Front Porch and Back Porch adjustments are located on the front of the right pedestal panel as shown in Figure 3.6-2.
       2) On non-ESP systems, the Front and Back Porch adjustments are located on the Sort Oscillator card:
          - Adjust R93 for the best side streams, while keeping the front porch level between 60% and 95% of the main pulse.
          - Adjust R92 for the best center (waste) stream while keeping the back porch level between 5% and 30% of the main pulse.

b. A change in sheath pressure may require you to readjust the front and back porch, even if you did not make any other adjustments.

Figure 3.6-2 ESP Front and Back Porch Adjustment Knobs



15. Check the side and center streams on the 1, 2, and 3 **Drops Sorted** settings.

    Note: The streams should look good on each of these settings. If not, you may have to either clean or change the flow cell.

16. Adjust the sort pulse shape after the best possible Sort Test streams are achieved.

    Note: You can observe the sort pulses with your scope using TP2 on the Sort Output card.

Delay Calculation

1. Ensure that the proper flow cell is selected on the cytometer Options screen. The flow cell options include:
    - 76 µm
    - 100 µm
    - Jet-in-air.
2. Calculate the droplet delay setting from the Camera screen of the Cytometer. Refer to the Sorting section of the Operator's Guide for instructions.
3. Go to the Sort screen.
4. Run the DNA Check beads to set a sort gate on a fluorescence signal.
5. Ensure that the **Droplets Sorted** is set to 1.
6. Set the **Sort Stop** to 100. This makes it easy to see the correct drop quickly in the fluorescent microscope.

7. Run a sort seven times: once for the actual Delay setting, three at less than the actual setting, and three at more than the actual setting on the Camera screen. Sort onto the slide at each setting.
8. View the sorted drops under the fluorescent microscope.
   - At least 90% of the beads should be in one drop. If so, select that drop and go to step 13.
   - If less than 90% of the beads are in one drop, go to step 9.
9. Select the drop that contains most of the beads.

   For example: If most of the beads are in drop 29, and some are in 30, then select 29 to be the drop of choice.
10. Place a cursor tangent to the tip of the drop; use the monitor to see the drop, and be sure that the camera view is zoomed in sufficiently to easily view the last drop and the filament connecting it to the stream.

ATTENTION: Make sure that the fluorescence signals do not drop when you raise the flow cell.

11. Turn the flow cell vertical adjustment to raise the flow cell and stream 1/16 of a drop. This makes the beads fall in the chosen drop (29 in the example).

    Note: This adjustment is more sensitive with quartz flow cell tips than with jet-in-air.
12. Sort the 100 beads using one delay before the chosen drop, the actual delay of the chosen drop, and one delay after the chosen drop.

    Note: Using the example in step 9 above, this means that you would choose 28, 29, and 30.
13. Check the drops on either side of your chosen drop.
    - If there are 10 beads or less in the drops on either side of the chosen drop, then there are 90 beads (90%) in the chosen drop. Go to step 14.
    - If there are 11 beads or more in the drops on either side of the chosen drop, then there are less than 90 beads in the chosen drop. Repeat steps 11 through 13.

    Note: Your adjustment depends upon which direction the beads fell. For example: If the flow cell was raised too much, the beads fell in the drop (28) before the chosen drop (29); if the flow cell was too low, the beads fell in the drop after (30) the chosen drop (29). If the flow cell was turned the wrong way, then most of the beads fell into the drop after (30) the chosen drop (29).
14. Reduce the number stored to 20.
15. Do a sort matrix on the phase of that droplet, which would be 0.0 to 0.9 on the delay.
16. Choose one of the phases and ensure that the side streams are good.

    Note: Several phases should have 20 in them.
17. Sort several drops of 20 particles onto a slide and use the fluorescent microscope to count so that you are absolutely sure of your delay and phase.
18. Make the camera delay match the actual delay by using the Flow Factor adjustment option on the Camera screen.

    Note: The system stores this value unless the software is reloaded, at which point it will have to be adjusted again for that particular flow cell.

Monitoring the Sort

1. Zoom in on the last attached droplet so that both the droplet position and connection neck are clearly visible.
2. Monitor stream stability:
   a. Place one cursor tangent to the bottom of the last attached drop.
   b. Place the other cursor tangent to the bottom of the second to the last attached drop.
3. View the side streams periodically while sorting to ensure that they are clean and stable.
4. Adjust the drive while watching the droplet in the camera to ensure the connection between the last drop and the second to last drop is not severed.

   Note: Optimal side streams are evident when the filament between the two drops is very thin and remains connected.
5. Ensure the side streams remain stable:
   - If the side streams remain stable, go to step 6.
   - If the side streams appear to slightly fan, adjust the crystal drive as follows:
     - If the filament connecting the last attached droplet breaks, decrease the crystal drive.
     - If the filament connecting the last attached droplet fattens, increase the crystal drive.
     - If the side streams remain unstable, stop the sort and investigate the problem.
6. Press **DEBUBBLE** and/or **CLEAR** three times.
   - If the droplet returns to the same place, the sort should be fine.
   - If the droplet does not return to the same place, then investigate further before proceeding with the sort, otherwise, the purity and recovery may be affected.
7. If you are performing this procedure in sequence beginning with Acquisition Check, return to step 7 under the Sort Check procedure.

PART B: OPTIONS AND UPGRADES INSTALLATION

3.7 OPTIONAL LASER INSTALLATION

Purpose

Perform this procedure to install an optional third laser, which can be:

- An Innova 305 laser
- An Omnichrome 356 laser
- An Innova 90 laser with remote box.

Currently Elite systems are shipped already equipped with the appropriate optical components, interlock components, and covers. As such, installation is limited to unpacking and mounting the optional laser system.

However, older Elite systems with optional lasers require installation of the cover kit, optics kit, and appropriate laser mounting kit.

Table 3.7-1 lists possible laser configurations.

Table 3.7-1 Possible Laser Configurations

Standard	Standard Plus One Water Cooled U.V. I305 Model	Standard Plus One Water Cooled U.V. 90-5 Model	Standard Plus One HeCd U.V. Series 74
1 air cooled Cyonics 488 laser	1 air cooled Cyonics 488 laser	1 air cooled Cyonics 488 laser	1 air cooled Cyonics 488 laser
1 HeNe 633 laser	1 HeNe 633 laser	1 HeNe 633 laser	1 HeNe 633 laser
	1 water cooled Innova 305 351.1 Coherent laser	1 water cooled 90-5 351.1 Coherent laser	1 HeCd U.V. Multimode 325 Omnichrome laser

Tools/Supplies Needed

- ❑ Kit for laser to be installed

Procedure

WARNING Risk of personal injury. Laser manufacturers of certain accessory lasers may require factory certification of the installer. Do not install any laser unless you have the required training and/or certification.

WARNING Risk of personal injury. The laser beam can cause eye damage if viewed either directly or indirectly from reflective surfaces (such as a mirror or shiny metal surface. To prevent eye damage, avoid direct exposure to the beam. Do not view it directly or with optical instruments except with special service tools as directed in this manual.

1. Determine which laser kits are present:

Kit	Part Number
Cover Upgrade	6912833
Optics	6912834
Laser Mounting	6912844 for Innova 305 laser 6912841 for 90-5 laser
Water cooled 305 laser	6912831
HeCd laser	6912801
Water cooled 90-5 laser	6912832

CAUTION Risk of instrument damage. The installation procedures shipped with the kits may contain the following errors:

- Installation Procedure, Optics, PN 9022633 Rev. A
  - Item 5 should be PN 6857612, 633 nm mirror
  - Item 4 should be PN 6857611, 488 nm mirror
- Installation Procedure, HeCd Model Kit, PN 9022632 Rev. A
  - Item 2 should be PN 2814049, 1/4 - 20 x 9/16 screw
  - Item 11 should be PN 6857611
  - Ext. Interlock Cable, PN 6028256, is needed but not shown

2. Examine the packing list for each kit to ensure that all parts are present, and notify shipping of any discrepancy.

   Note: An installation procedure should accompany all kits.

3. Inspect all optional lasers for correct operation. For specifications and procedures, refer to the manual(s) supplied with the laser.
4. Determine the revision level of the Dual Laser Controller card in the Elite.
   - If the revision level is not D or later, order a replacement card immediately.
   - If the revision level is D or later, go to step 5.
5. Remove all covers from the optical bench and laser areas.
6. Set the end panel in place temporarily.

   Note: The water cooled laser does fit through the padded opening.

7. Using Figure 3.7-1 as a guide:
   a. Route the interlock cable attached to the panel down to the Multibus area.
   b. Attach the relay bracket to the Multibus card cage.
8. Wire all the connections as shown in Figure 3.7-2.

Figure 3.7-1 Path of Interlock Cable to Multibus System

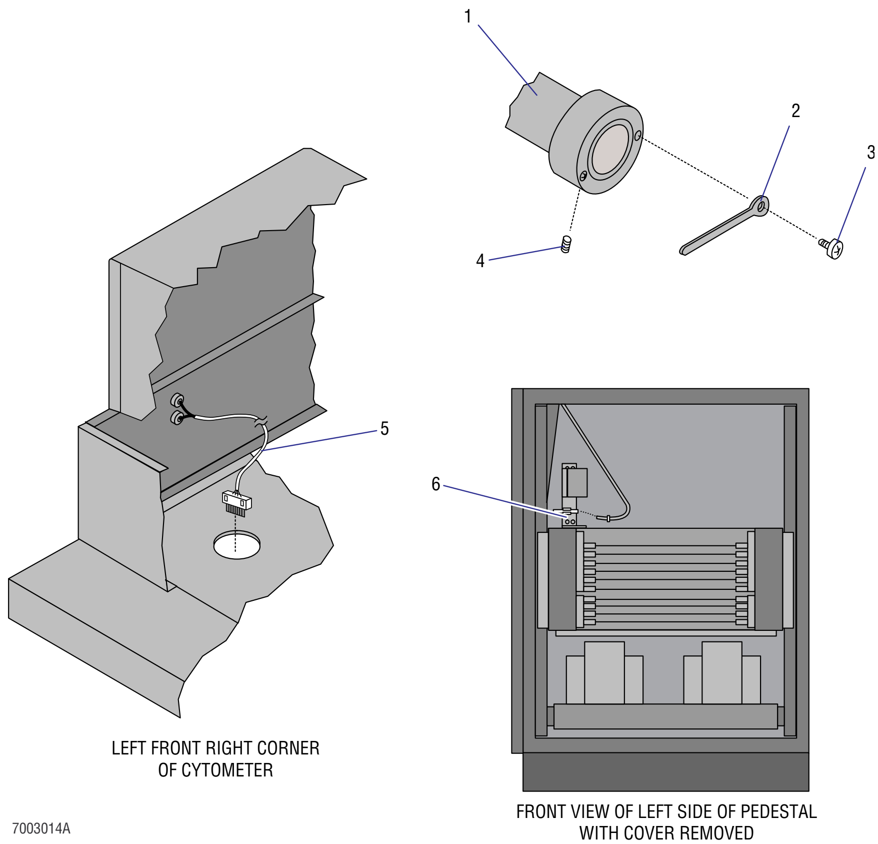
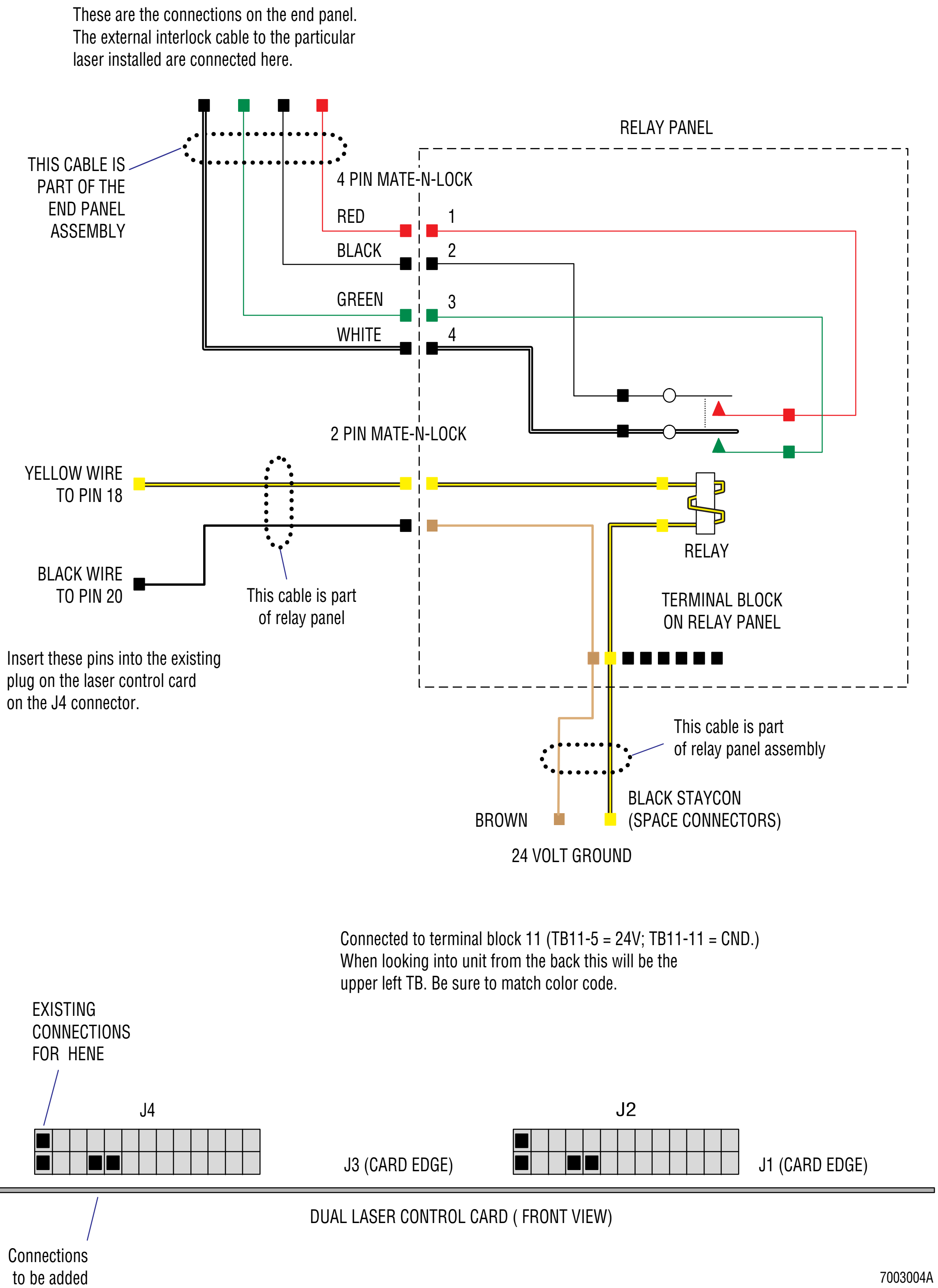
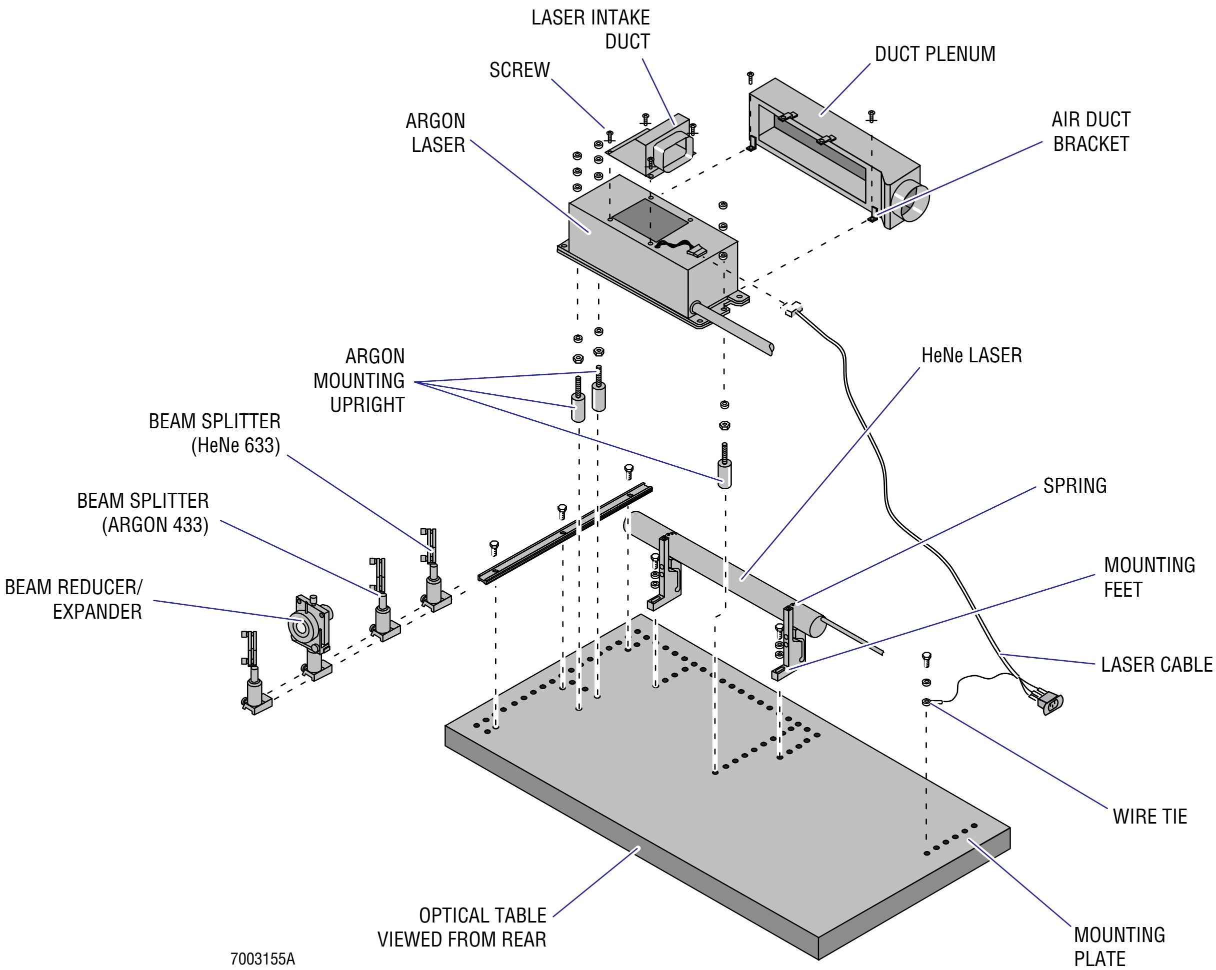


Figure 3.7-2 Cable Connections for Laser Installation



9. Install the Argon laser as shown in Figure 3.7-3:
    a. Move the Argon laser.
    b. Install the rail on the optical table.
    c. Connect the new external blower.

Figure 3.7-3 Argon Laser Installation



10. Target the HeNe laser and air cooled Argon lasers:
    a. Insert the tall targets and target the HeNe and Argon lasers.
    b. Remove the tall targets.
    c. Insert the short targets in place of the scatter sensor and the beam shutter, and target the lasers beginning with the laser closest to the sampling compartment.
    d. Remove the short targets.
11. Remove the flow cell tip.
12. Place the HeNe mirror in front of the HeNe laser.
13. Place the 488 mirror in front of the Argon laser.
14. Use the mirror mount adjustments to align the two lasers through the targets:
    a. Loosen both the rail setscrew and the fine adjustment setscrew.

b. Slide the mount to the approximate location desired.

c. Tighten the rail setscrew just enough to keep the mount from wobbling but still allowing it to slide on the rail.

d. Tighten the fine adjustment setscrew; be careful not to overtighten.

e. Use the fine adjustment knob to make the final location adjustment.

f. Tighten the rail setscrew to lock the mount in place; be careful not to overtighten.

WARNING Risk of personal injury. The HeCd and water cooled Argon lasers operate in the UV (ultraviolet) mode which can injure you when exposed. Use extreme care to avoid exposure. Carefully note the probable beam paths before firing the laser, and block the beam from exiting the instrument by using a piece of white paper, such as a business card, to locate the beam during alignment.

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15. Install the HeCd or water-cooled Argon laser. Refer to the appropriate figures for the laser being installed:

    a. HeCd Laser

    - Figure 3.7-4, HeCd Laser Head Mounting
    - Figure 3.7-5, HeCd Power Supply Connections
    - Figure 3.7-6, HeCd Optical Arrangement
    - Figure 3.7-7, HeCd Laser Configuration

    b. Water-cooled Laser

    - Figure 3.7-8, I305 Mounting
    - Figure 3.7-9, Water-Cooled Optical Arrangement
    - Figure 3.7-10, Water-Cooled Laser Arrangement

Figure 3.7-4 HeCd Laser Head Mounting

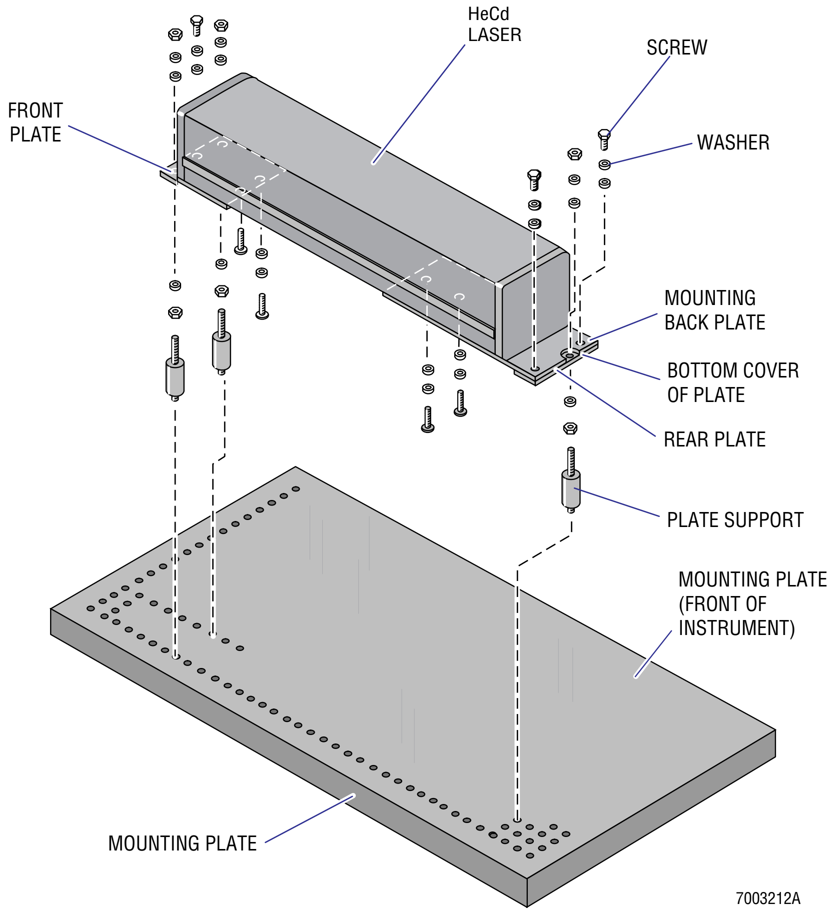


Figure 3.7-5 HeCd Power Supply Connections

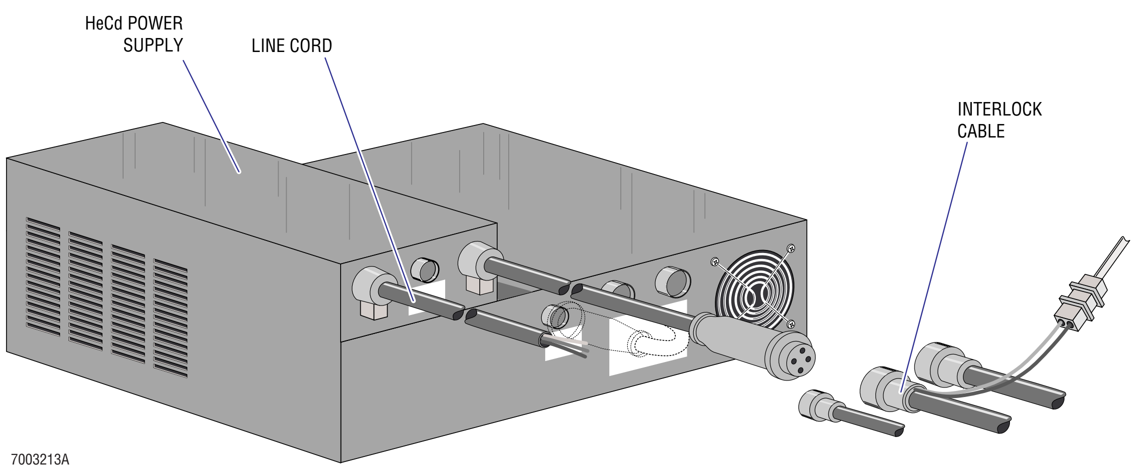


Figure 3.7-6 HeCd Optical Arrangement

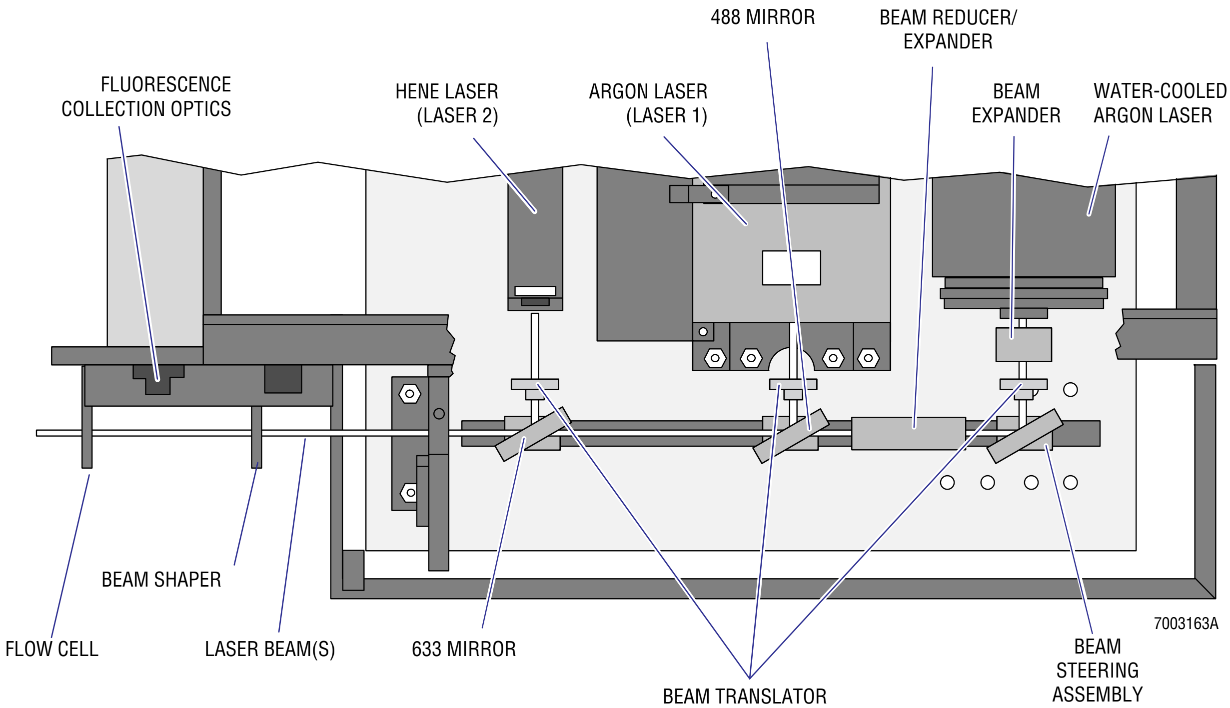


Figure 3.7-7 HeCd Laser Configuration

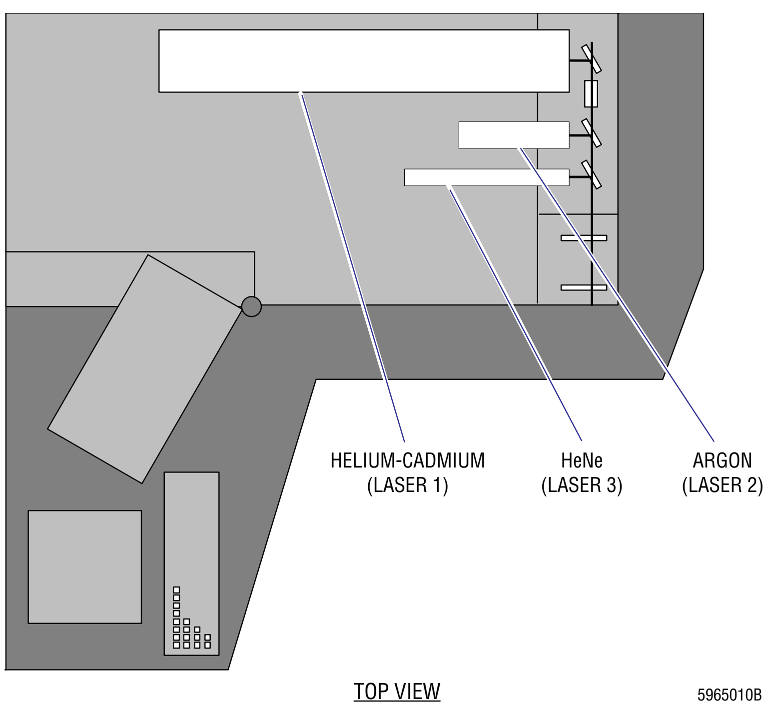


Figure 3.7-8 I305 Mounting

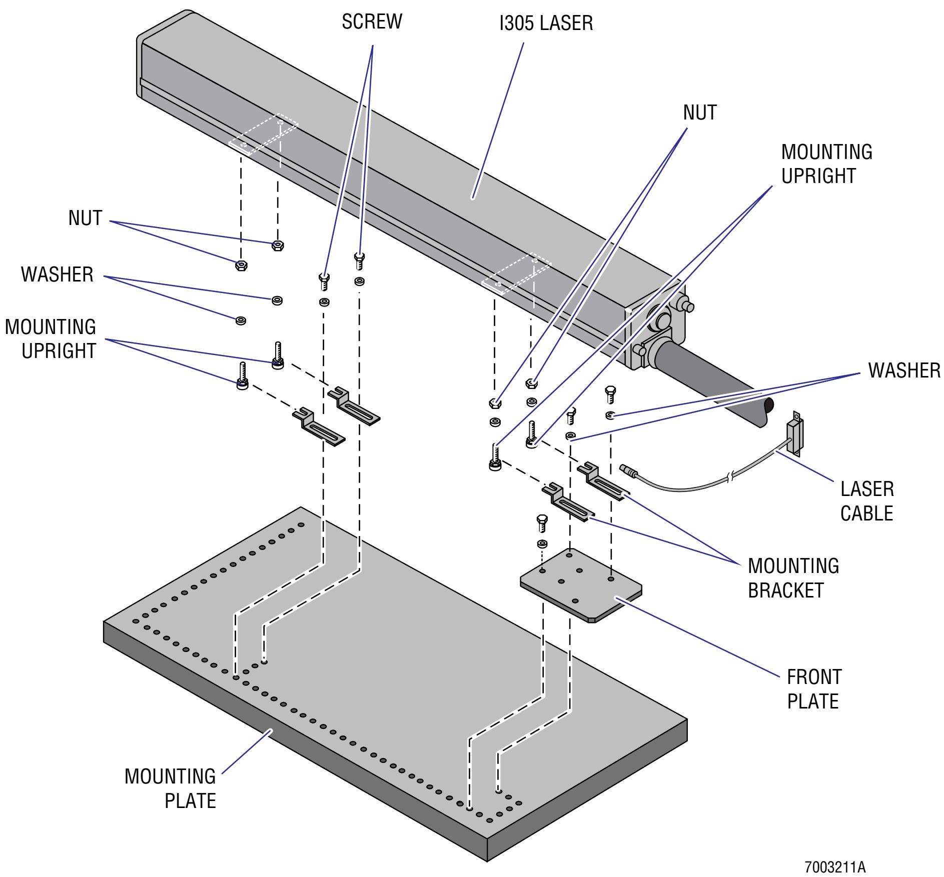


Figure 3.7-9 Water-Cooled Optical Arrangement

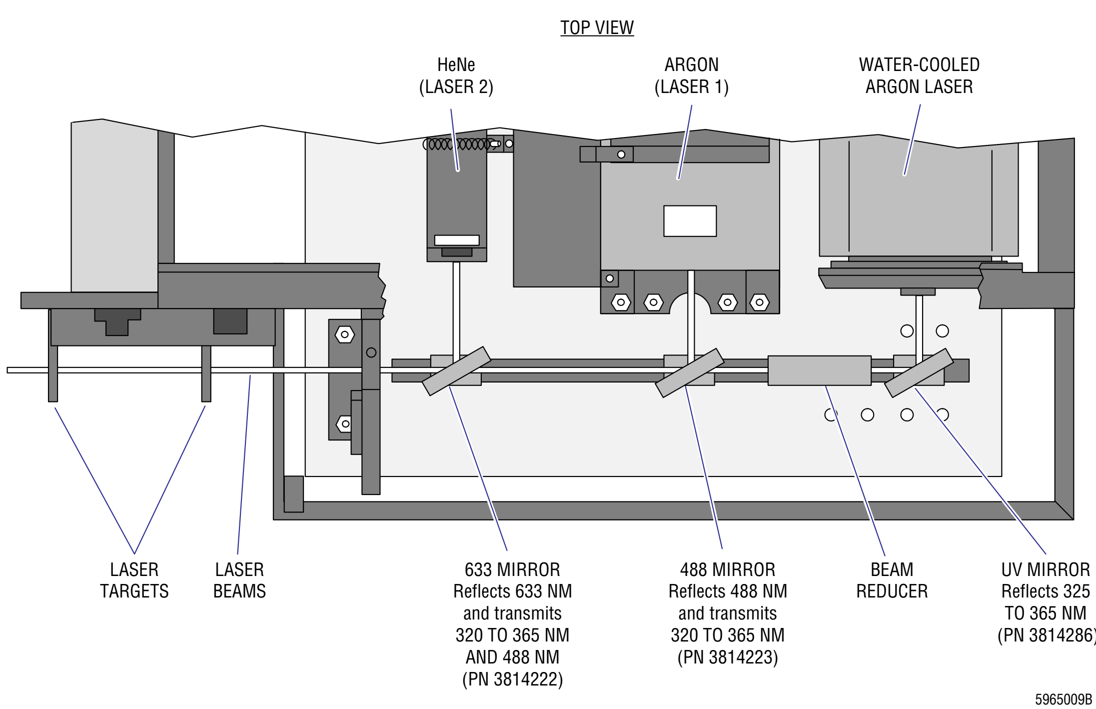
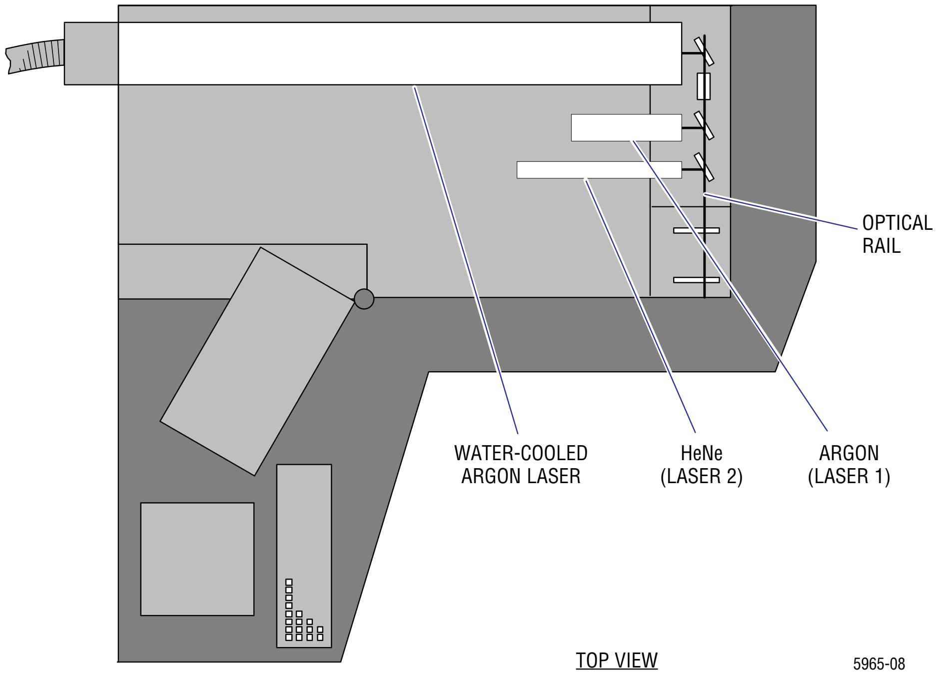


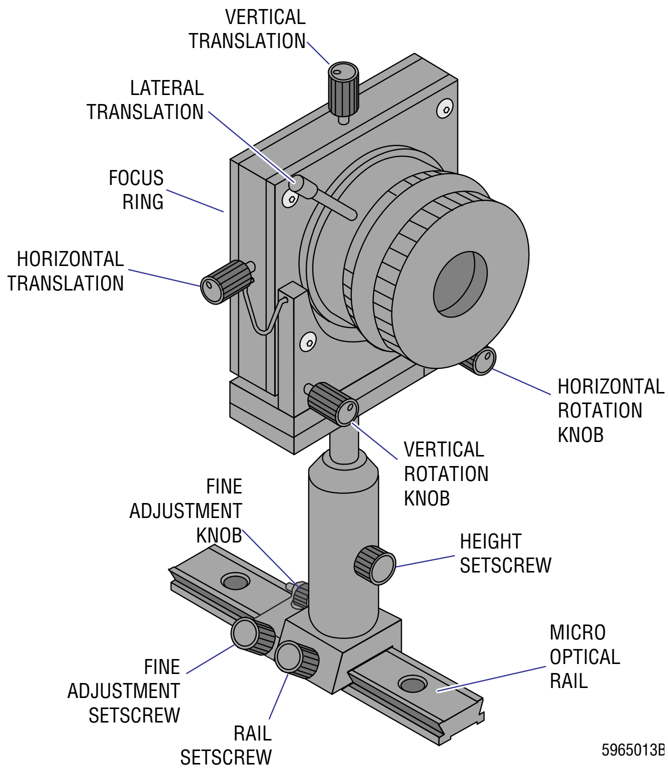
Figure 3.7-10 Water-Cooled Laser Arrangement



WARNING Risk of personal injury. The HeCd and water-cooled Argon laser will be operated in the UV mode which can injure you when exposed. Use extreme care to avoid exposure. Carefully note the probable beam paths before firing the laser, and block the beam from leaving the instrument. Use a white piece of paper (such as a business card) to locate the beam during alignment.

16. Use the **tall** targets to align the UV laser to the optical table.
17. Secure the laser in place with the clamps provided.
18. Remove the tall targets.
19. Install the UV mirror assembly on the rail. Use the mirror adjustments to target the UV laser through the small targets.
20. Install the beam expander (Figure 3.7-11) between the UV laser and the air-cooled Argon laser and align it with the small targets.
    - The beam entering the expander will be about 0.3 mm in diameter.
    - The beam leaving the expander will be about 0.15 mm in diameter just before it passes through the HeNe mirror.

Figure 3.7-11 Beam Expander/Reducer



21. Remove targets and replace the FALS sensor, flow cell tip, and shutter.
22. Use the camera to align the flow cell.
23. Run fluorospheres.
24. Align the beam shaper for optimal signals on FALS and PMT 4 with the HeNe.

    Note: Remove the 675 BP and the ND filter. On 5 PMT systems, also remove the 640 DL.
25. Block the HeNe beam to allow the air-cooled Argon beam to pass.
26. Adjust the air-cooled mirror for optimal FALS signals. Note the width of the PMT 2 Peak pulse.
27. Open the HeNe and align the air-cooled mirror to place the Argon beam on top of the HeNe with FALS or PMT 2 Peak and PMT 4 Peak.

ATTENTION: Do not adjust the beam shaping optics.

28. Carefully remove the beam reducer (Figure 3.7-11).
29. Block the HeNe and air-cooled Argon beams.
30. Align the UV mirror to obtain optimal signals on PMT 2.

    Note: The beam is out of focus at this point so the signal will be bad.
31. Install the beam reducer and adjust it for optimal signals from PMT 2 Peak. Adjust the beam reducer focus ring to match the pulse width with the Argon pulse as measured in step 26.

32. Open the HeNe beam and use only the beam reducer and the PMT 2 Peak and PMT 4 (or PMT 5 on 5 PMT systems) Peak adjustments to align the UV beam to be on top of the HeNe beam.
33. Install remaining covers. See Figure 3.7-12 and Figure 3.7-13.
34. Install warning labels. See Figure 3.7-14 and Figure 3.7-15.

Figure 3.7-12 HeCd Laser Covers

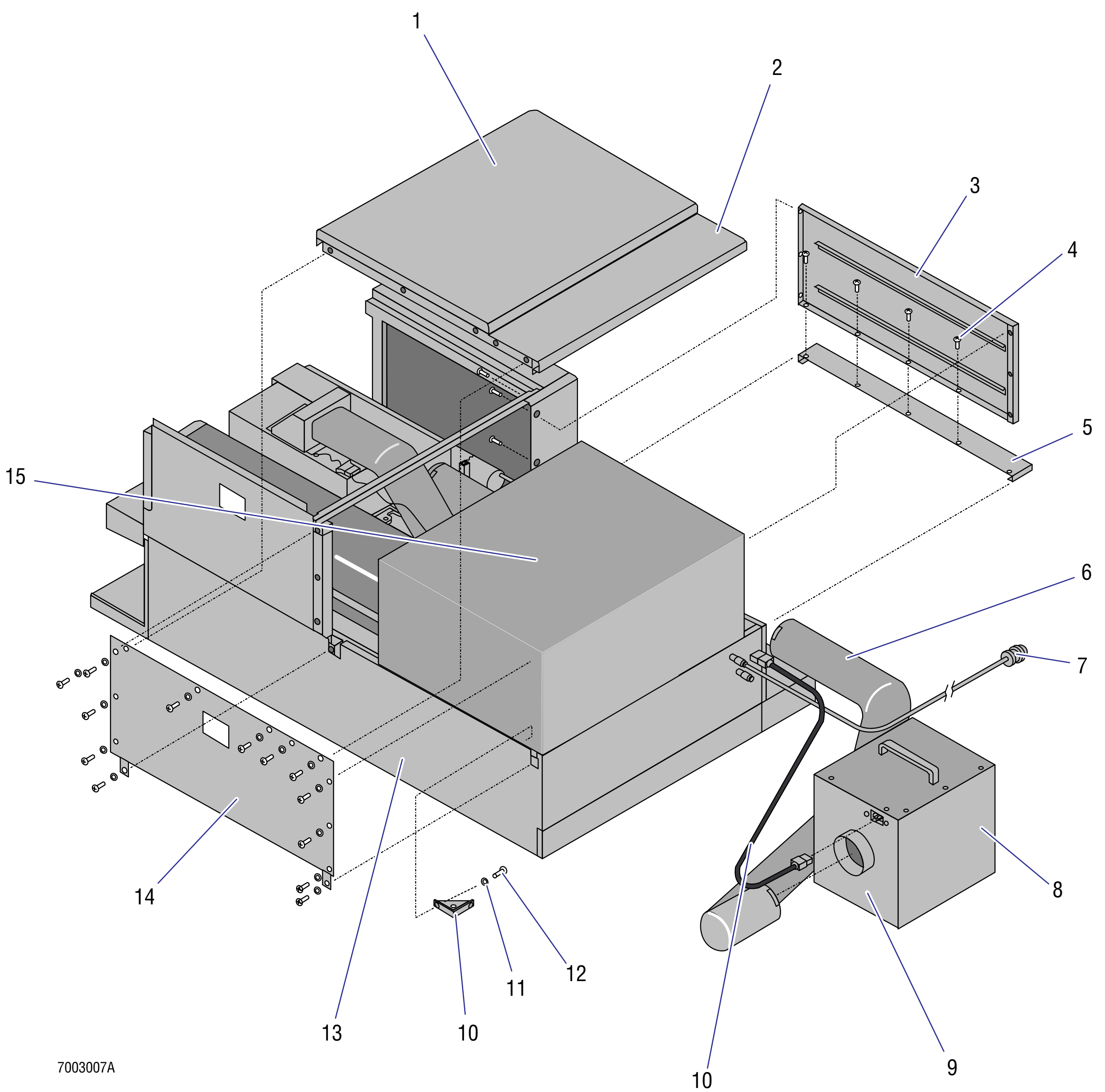
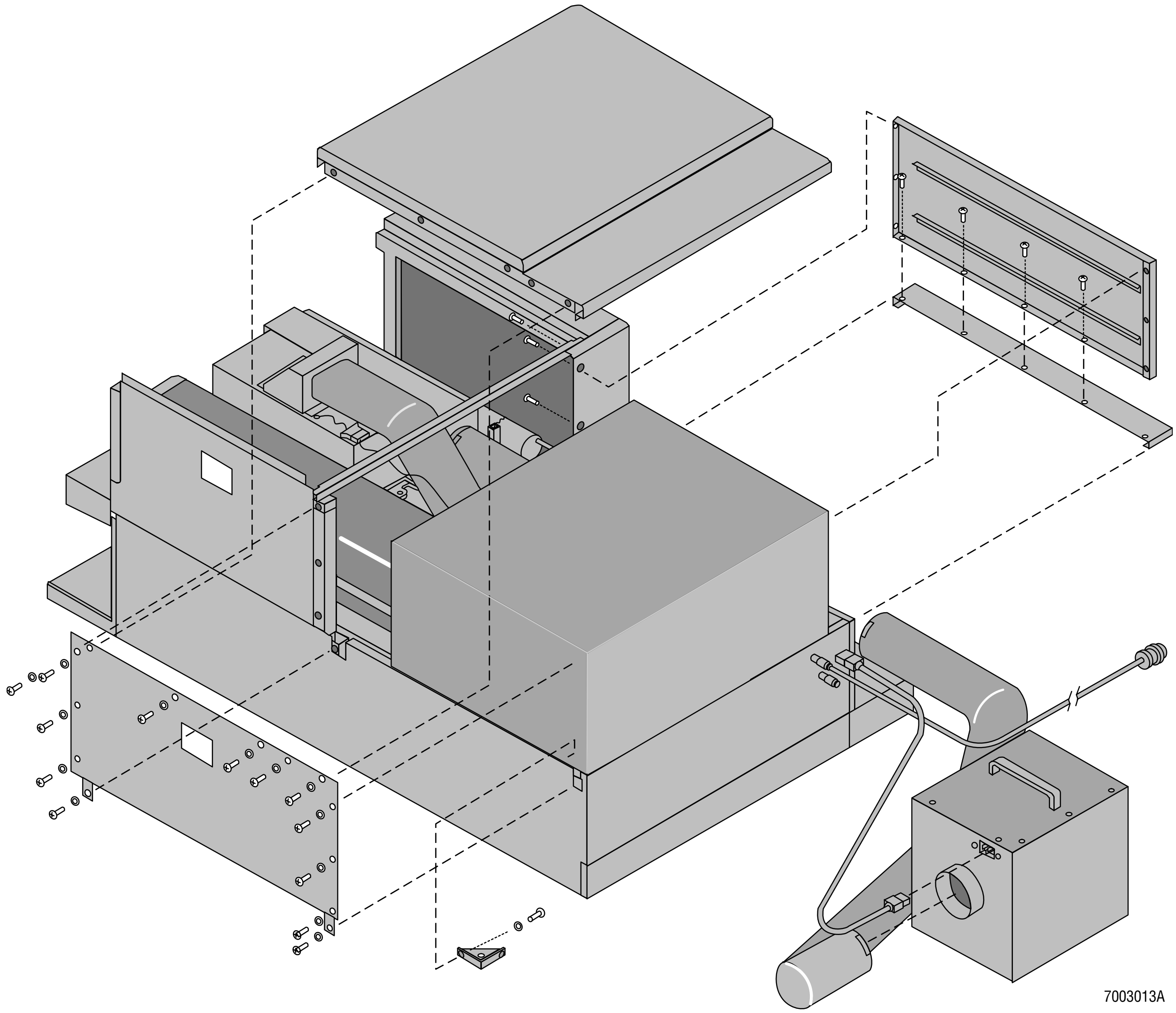


Figure 3.7-13 Water-Cooled Laser Covers



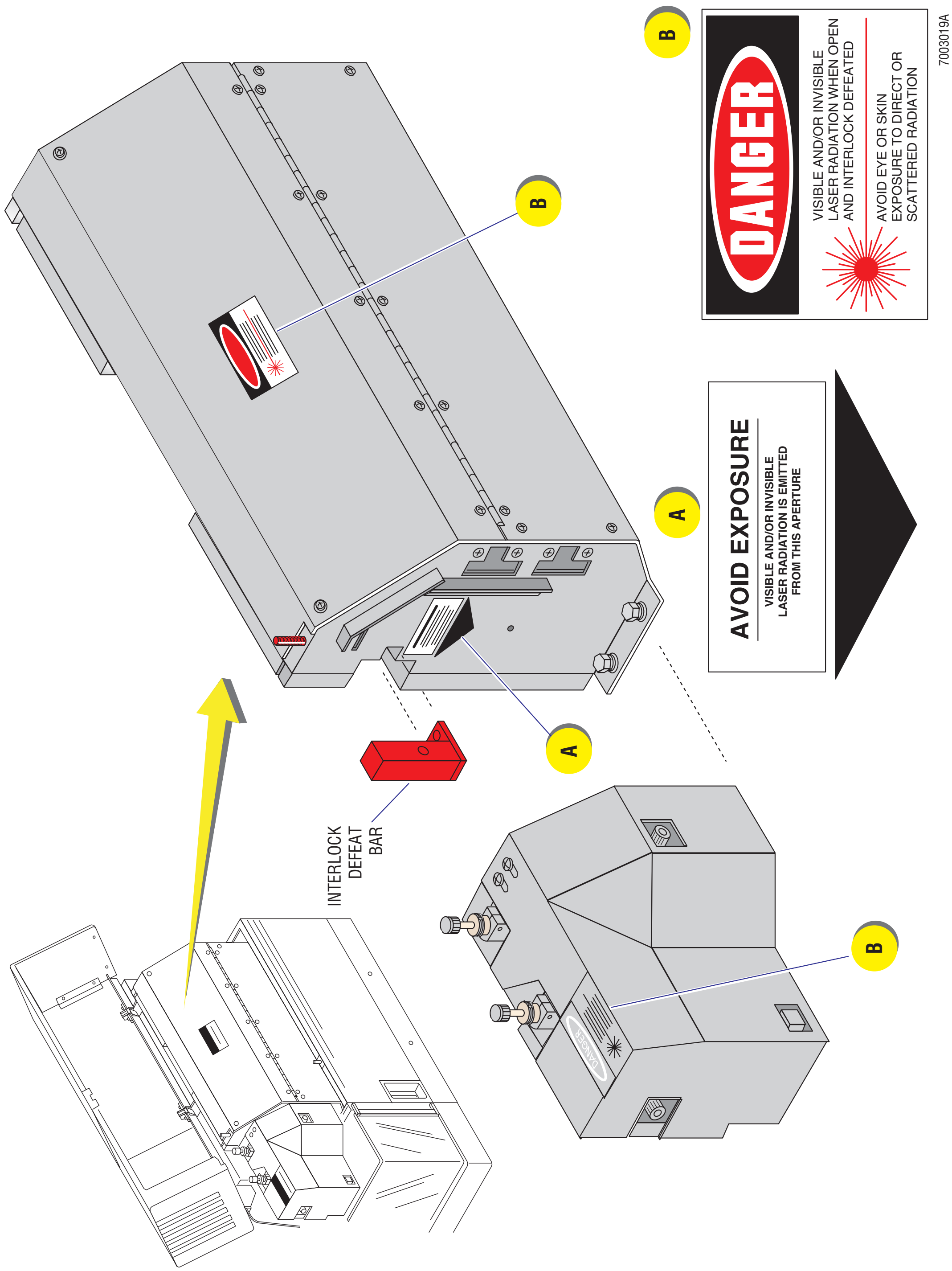


Figure 3.7-14 Warning Labels in Sensing Area

Figure 3.7-15 Warning Labels, Rear of Instrument

35. Verify interlock operation by interrupting the optical area safety cover.
    a. Verify that all covers are installed.
    b. Turn on the Cytometer.
    c. Power up Laser 1, Laser 2, and appropriate optional laser. **Result:** All three lasers should turn on.
    d. Lift open optical area safety cover. **Result:** All three lasers should shut down.
    e. Turn on all three lasers. **Result:** Lasers should not turn on with cover open.
    f. Verify that the certification label and rear cover warning label are in place.

    Note: Ensure that the external blower draws cooling air through the laser head when the laser is turned ON.
36. Replace covers before resuming operation.

3.8 HOUSE AIR INSTALLATION

Procedure

1. Turn the instrument off.
2. Open the pneumatic housing door.
3. Remove the four screws securing the bottom side cover and remove the cover.
4. Open the bottle tray door and disconnect unit system air quick-connect QD22 (blue) and system vacuum QD23 (yellow) from compressor. Disconnect the white connector (not used with this kit).
5. Detach the compressor from support shipping brackets (if attached) and remove compressor from the pneumatic housing.
6. Remove the Compressor shipping bracket located below valve bracket
7. Attach gauge and regulator box inside pneumatic housing, at the compressor shipping bracket location.

   Note: Use the same shipping bracket screws for the installation or the new screws provided.
8. Ensure the box system pressure regulator knob is rotated fully counterclockwise.
9. Connect QD22 (blue) and QD23 (yellow) into corresponding box connectors system air and system vacuum.
10. Connect orange and white quick-connects to the same color body couplings of the box called house air and house vacuum.
11. Attach the two air and vacuum lines to the customer air and vacuum system to be used. Split into two lines (vacuum and air) to suit customer's needs.
12. Turn unit power ON.
13. Ensure the system pressure gauge does not exceed 30 psi and that the system vacuum gauge indicates at least -10 in. Hg.
14. Adjust the regulator to ensure 30 psi on the gauge during operation.

3.9 GATED AMPLIFIER UPGRADE

Purpose

Use this procedure to install the Gated Amp kit in the Elite or Elite Analyzer. Instruments ordered with a Gated Amp configuration are shipped with it already installed.

The Gated Amp (GA) consists of two main sections: a Switchable Amp section and a Gated Amp section. The new amplifiers allow high speed/low speed switching while the Peak Signal Boards allow the acquisition of FALS and PEAK signals, in addition to enhancing the control of the Discriminators SAT function.

The following are prerequisites to installing the Gated Amp kit:

- Software revision 3.0 or above.
- External Memory Upgrade in the Cytometer Multibus card cage.

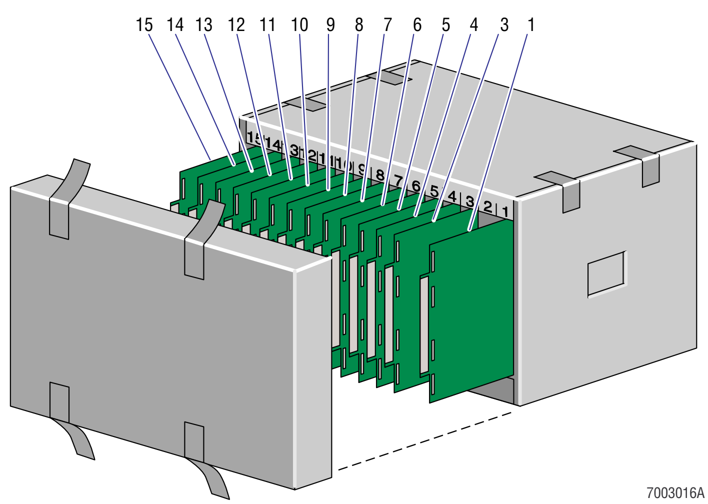
  Note: If this upgrade is needed, see Heading 4.13 for instructions.

The Gated Amp Kit is wired and packed in an anti-static container (Figure 3.9-1). The container is specifically made for ease of installation. Cards are pre-wired within the container and are ready to slide into the top card rack one at a time interconnected to each other. The container is packed in a brown cardboard box. The height of the cardboard box is approximately the same as the bottom lip of the top card rack.

Figure 3.9-1 Anti-Static Container of Gated Amp Kit

LEGEND

SLOT #	CARD NAME
1	Gated Amp Control R 3
2	
3	Dual FL SW-R
4	3 PMT SUB SW-R2
5	3 PMT SUB SW-R1
6	PMT Gated Amp
7	Quad 20/40/60 Microsecond Delay 2
8	Quad 7 Microsecond Delay 2
9	Quad 7 Microsecond Delay 1
10	Quad 20/40/60 Microsecond Delay 1
11	Scat / Aux Gated Amp
12	3 PMT SUB SW-R
13	Peak Scatter / Mux SW-R
14	Scat / CV SW-R1
15	Mux and Scope Interface



Tools/Supplies Needed

- ❑ C-type extender card, PN 6702299
- ❑ One of the following kits:
  - ▸ Gated Amp Service Field Upgrade Kit (for units before SN U07122) PN 6912827
  - ▸ Gated Amp Service Field Upgrade Kit (for units after SN U07122) PN 6912798
- ❑ Alignment Tool, PN 5402071
- ❑ Oscilloscope

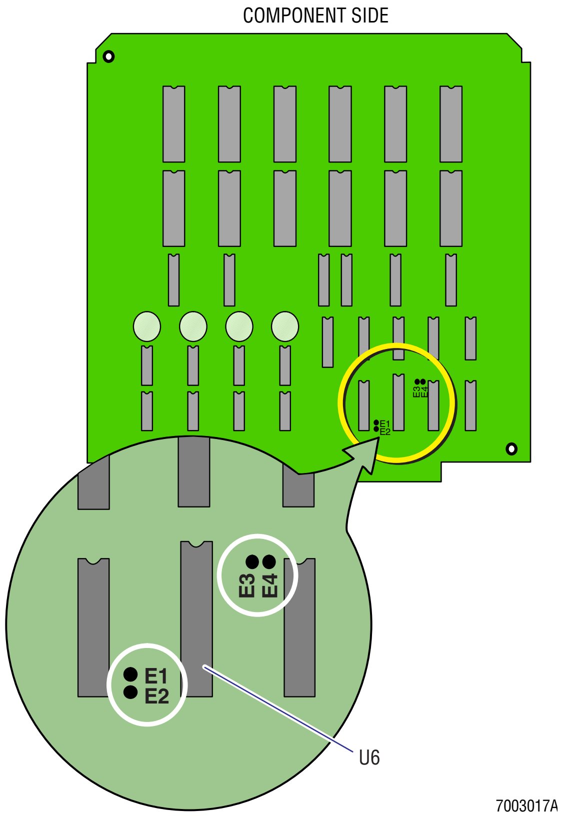
Procedure

1. Examine the packing list for the kit and ensure all parts are present. Notify shipping of any discrepancy.
2. Verify operation of all signals and unit function before beginning kit installation.
3. Turn OFF power at Cytometer and Workstation.
4. Remove the tabletop.
5. Ensure cables from PMTs and FALS sensor are labeled.
6. Remove the existing Scatter Sensor R Pre-Amp card and replace with the new Peak Scatter Sensor card, connecting P105 to J35 and P83 to J36.
7. Reinstall the forward scatter sensor assembly.
8. From the top card cage, counting from the left, disconnect the coax cables from the following cards and remove the cards:
   - Slot #1 Gated Amp Control R2
   - Slot #6 3 PMT Sub Amp R2
   - Slot #7 3 PMT Sub Amp R1
   - Slot #8 Dual FL Amp R1
   - Slot #9 Scat/CV Amp R.
9. Apply the new slot identification label over the old label on top of card cage.

   Note: Slot 1 is on the left.
10. Rest the anti-static container on the top card rack.
11. Remove exiting Mux and Scope Interface card, slot 6.

    Note: The Mux and Scope card is installed in the container in slot 15. Remove the Mux and Scope Card from the container, but leave it attached to facilitate the sliding of the cards into their respective slots.
12. Install the Mux and Scope card last in slot 6 of the bottom card cage.
13. Verify jumpers E1-E2 and E3-E4 are in place. See Figure 3.9-2.

Figure 3.9-2 Jumpers, Mux and Scope Card



14. Verify that PAL chip U6 PN 6704597 is installed.
15. Connect the upper trace (UT) coax to Mux and Scope J29.
16. Connect the lower trace (LT) coax to Mux and Scope J30.
17. Connect the small blue ribbon cable from Mux and Scope J27 to Digiscope J1.
18. Determine if the kit is pre-wired:
    - If the kit is pre-wired, install the kit and go to step 22.
    - If the kit is not pre-wired, go to step 19.
19. Install the new Gated Amp Control R3 card in first slot from left in the top cage.
20. Install the new Dual FL SW-R Card in third slot from left in the top cage, and jumper as #1, E1-E2 Out.
21. Install the new 3 PMT Sub SW-R card:
    a. 4 PMT: Jumper as #1, E1-E2 OUT, E3-E4 OUT, E5-E6 OUT, and install in fourth slot from left in the top cage.
    b. 5 PMT: Jumper as #2, E1-E2 OUT, E3-E4 IN,E5-E6 OUT, and install in fourth slot from left in the top cage.

22. See Table 3.9-1 and jumper:
    a. The new 3 PMT Sub SW-R card #1: E1-E2 OUT, E3-E4 OUT, E5-E6 OUT, and install in fifth slot from left in the top cage.
    b. The new PMT Gated Amp R card as #1, E1-E2 OUT, and install in sixth slot from left in the top cage.
    c. The new Quad 20/40/60 Microsecond Delay card as #2, E1-E2 IN, E3-E4 IN, E10-E11 IN, and install in seventh slot from left (top cage).
    d. The new Quad 7 Microsecond Delay card as #2, E10-E15, and install in 8th slot from left in the top cage.
    e. The new Quad 7 Microsecond Delay card as #1, E10-E11 IN, and install in 9th slot from left in the top cage.
    f. The new Quad 20/40/60 Microsecond Delay card as #1, E1-E2 IN, E3-E4 IN, E9-E10 IN, and install in 10th slot from left in the top cage.
    g. The new Scat Aux Gated Amp R card as #1, E2-E3 OUT, and install in 11th slot from left in the top cage.
    h. The new 3 PMT Sub SW-R card as #3, E1-E2 IN, E3-E4 OUT, E5-E6 IN, and install in 12th slot from left in the top cage.
    i. The new Peak Scatter/Mux SW-R card as E1-E2 IN, E3-E4 IN, and install in 13th slot from left in the top cage.
    j. The new Scat/CV SW-R card as #1, E1-E2 OUT, E3-E4 OUT, and install in 14th slot from left in the top cage.
    k. The new Mux and Scope card.

Table 3.9-1 Gated Amp Jumper Configuration

Circuit Card	Slot	Jumpers
Gated Amp Control R 3	1	None
Dual FL Switch-R	3	E1-E2 OUT
3 PMT Sub W-R 2	4	5 PMT: E1-E2 OUT, E3-E4 IN, E5-E6 OUT 4 PMT: E1-E2 OUT, E3-E4 OUT, E5-E6 OUT
3 PMT Sub SW-R1	5	E1-E2 OUT, E3-E4 OUT, E5-E6 OUT
PMT Gated Amp	6	E1-E2 OUT
Quad 20/40/60 Microsecond Delay 2	7	E1-E2 IN, E3-E4 IN, E10-E11 IN
Quad 7 Microsecond Delay 2	8	E10-E15 IN
Quad 7 Microsecond Delay 1	9	E10-E11 IN
Quad 20/40/60 Microsecond Delay 1	10	E1-E2 IN, E3-E4 IN, E9-E10 IN
Scat/Aux Gated Amp	11	E2-E3 OUT
3 PMT Sub SW-R 1	12	E1-E2 IN, E3-E4 OUT, E5-E6 IN
Peak Scatter/Mux Sub SW-R	13	E1-E2 IN, E3-E4 IN
Scat/CV SW-R 1	14	E1-E2 OUT, E3-E4 OUT

23. Install the Mux and Scope card in slot 6 of the bottom card cage.
24. Connect cards with coax cables as indicated in Table 3.9-2.

Table 3.9-2 Coax Cable Interconnections

From	To
Quad 7 Microsecond Delay 2 (slot 8) J2 J4 J6 J8	Quad 20/40/60 Delay 2(slot 7) J2 J5 J8 J11
Quad 20/40/60 Microsecond Delay 2 (slot 7) J4 J7 J10 J3 J1 J6 J9 J12	PMT Gated Amp (slot 6) J9 J10 J11 J13 J8 J14 J17 J18
Quad 7 Microsecond Delay 2 (slot 8) J1 J3 J5 J7*	PMT Gated Amp (slot 6) J12 J15 J16 J19
Quad 7 Microsecond Delay 1 (slot 9) J8 J6 J4 J2	Quad 20/40/60 Delay 1 (slot 10) J11 J8 J5 J2
Quad 20/40/60 Delay 1(slot 10) J1 J4 J7 J10 J3 J6 J9 J12	Scat/Aux Gated Amp (slot 11) J6 J7 J8 J9 J11 J12 J15 J16
Quad 7 Microsecond Delay 1 (slot 9) J1 J3 J5 J7*	Scat/Aux Gated Amp (slot 11) J10 J13 J14 J17
PMT Gated Amp (slot 6) J20 J21 J22	3PMT Sub SW-R 1 (slot 5) J3 J2 J1

**Bundle these cords and continue.*

Table 3.9-2 Coax Cable Interconnections

From	To
PMT Gated Amp (slot 6) J23 J24 J25	3PMT Sub SW-R 2 (slot 4) J1 J2 J3
3 PMT Sub SW-R 1 (slot 5) J5	Dual FL SW-R (slot 3) J1
3 PMT Sub SW-R 2 (slot 4) J5 (5 PMT Installation) J4 (4 PMT Installation)	Dual FL SW-R (slot 3) J5 J5
Scat/Aux Gated Amp (slot 11) J18	Peak Scatter/Mux SW (slot 13) J11
Scat/Aux Gated Amp (slot 11) J19 J20 J21	3PMT Sub SW-R3 (slot 12) J1 J2 J3
3 PMT Sub SW-R3 (slot 12) J5 J6 J7 J8	Peak Scatter/Mux SW (slot 13) J1 J8 J6 J5
3PMT Sub SW-R 3 (slot 12) J4	Scat/CV SW-R1 (slot 14) J1
Peak Scatter/ Mux SW (slot 13) J2 J3	Scat/CV SW-R1 (slot 14) J6 J5
3 PMT Sub SW-R 1 (slot 5) J6	Peak Scatter/Mux SW (slot 13) J9
Dual FL SW-R (slot 3) J6 J6	Scat/CV SW-R1 (slot 14) J8
PMT Gated Amp (slot 6) J2 J3	Scat/Aux Gated Amp (slot 11) J4 J5
Peak Scatter/Mux SW (slot 13) J12	Scat/CV SW-R1 (slot 14) J10
Scat/Aux Gated Amp (slot 11) J1	Gated Amp Control R3 (slot 1) J3
PMT Gated Amp (slot 6) J1	Gated Amp Control R3 (slot 1) J4

**Bundle these cords and continue.*

Table 3.9-2 Coax Cable Interconnections

From	To
Mux and Scope J1 J2 J3 J4	PSH 1 (slot 7) J1 J3 J5 J7
PSH 1 (slot 7) J5 J6 J7 J8	PSH 2 (slot 8) J1 J3 J5 J7
Mux and Scope J29	Lower Trace (LT)
Mux and Scope 30	Upper Trace (UT)
Mux and Scope J26	No Connection
Mux and Scope J10	Scat/CV SW-R 1 (slot 14) J4
Mux and Scope J11 J12	Scat/CV SW-R 1 (slot 14) J3 J2
Mux and Scope J13 J14	3PMT Sub SW-R 1 (slot 5) J8 J7
Mux and Scope J15 J9	Peak Scatter Mux SW (slot 13) J4 J14
Mux and Scope J16 J17	Dual FL SW-R (slot 3) J3 J4
Mux and Scope J18 J19 J20	Dual FL SW-R (slot 3) J2 J7 J8
Mux and Scope J21	Scat/CV SW-R1(slot 14) J9
Mux and Scope J22 J23	3PMT Sub SW-R2 (slot 4) J8 J7
Mux and Scope J24	Peak Scatter Mux SW-R (slot 13) J10

**Bundle these cords and continue.*

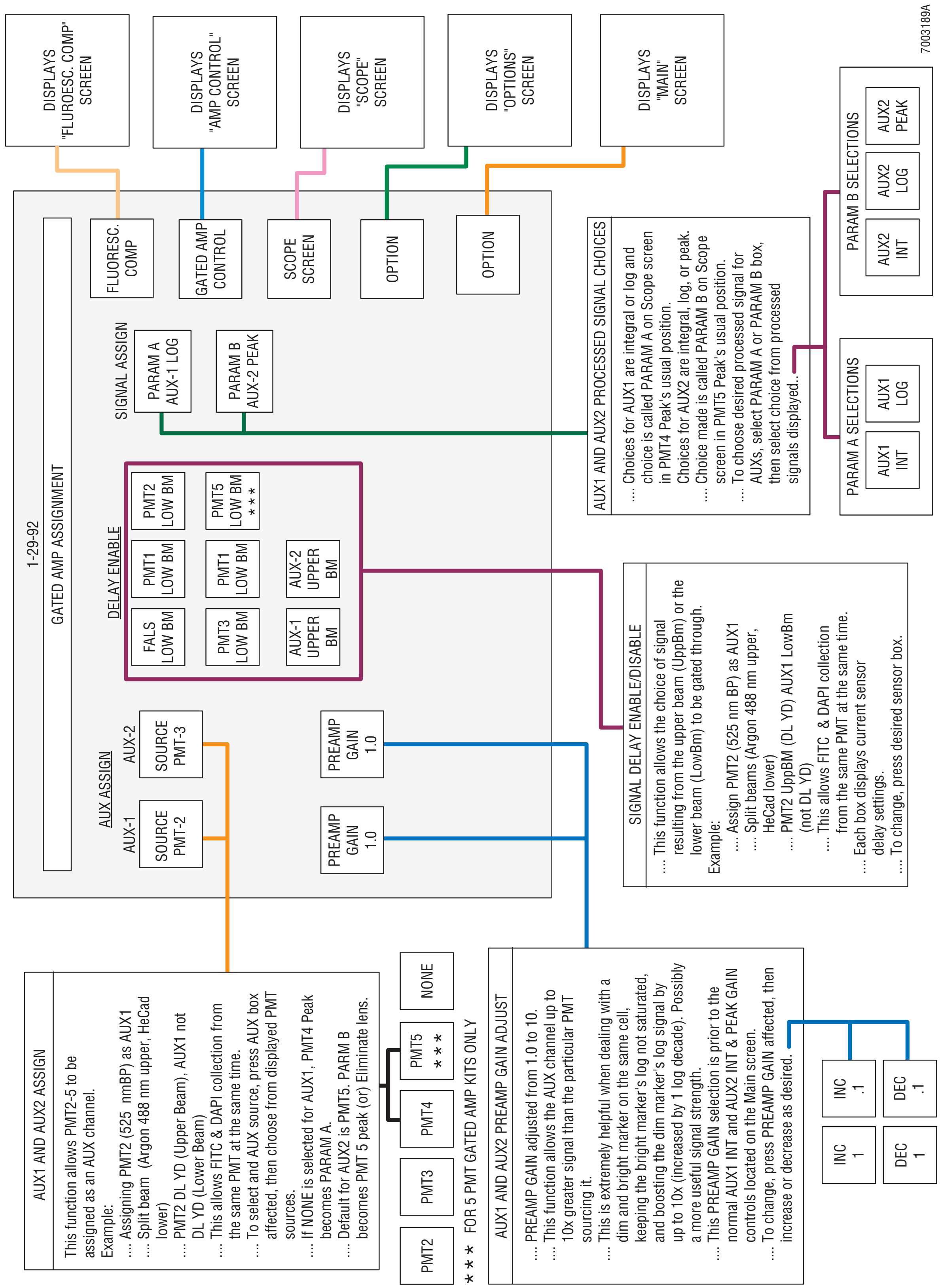
Table 3.9-2 Coax Cable Interconnections

From	To
Mux and Scope J25	Gated Amp Control R3 (slot 1) J2
Mux and Scope J31	Gated Amp Control R3 (slot 1) J6
Interconnections from Sensor Area	
FALS	Scat/Aux Gated Amp (slot 11), J2
PMT1	Scat/Aux Gated Amp (slot 11), J3
PMT2	PMT Gated Amp (slot 6), J4
PMT3	PMT Gated Amp (slot 6), J5
PMT4	PMT Gated Amp (slot 6), J6
PMT5 (If present)*	PMT Gated Amp (slot 6), J7

**Bundle these cords and continue.*

25. Remove the Quad 20/40/60 Microsecond Delay card from slot 10 and put into the extender.
26. At the Cytometer:
    a. Go to the Scope screen and set **TIME/DIV** to 10 msec/div.
    b. Go to the Sort screen and set the **Frequency** to 2 kHz and turn **Strobe** on.
    c. Open the detector compartment and remove the 488 BP filter.
    d. Set the Lower Trace to PMT1 INT and the Upper Trace to FALS INT.
27. Move the deflection body all the way up and remove the FALS filter.
28. Adjust the PMT1 gain to 10 and the HV gain for about a three-division high PMT1 pulse.
29. Adjust the FALS INT gain and HV to produce a three-division high FALS signal. The FALS signal should be clean on the upper trace.
30. Close the detector compartment door.

Figure 3.9-3 Gated Amplifier Assignment Screen



FALS Adjustment: Assignment and Adjustment Potentiometer Location

Note: When in the delay Enable section of the Gated Amp Assignment screen (Figure 3.9-3), LWBM means no delay, and UPBM means delay is on. See Table 3.9-3 and Figure 3.9-4 for additional information on adjustments.

1. At the Gated Amp Control screen (Figure 3.9-5):
   a. Ensure FS INT is on the top trace and PMT1 INT is on the bottom trace. Note amplitude of FS INT on scope upper trace (3 divisions).
   b. Change the delay to 7 microseconds, then go to the Gated Amp Assignment screen (Figure 3.9-6).
   c. Select **FALS** for the **UPBM** and record the amplitude of the FALS trace, on Scope upper trace.
2. Adjust R105 on the Quad 7 Delay #1, slot 9, so the delayed pulse has the same amplitude as the original FALS pulse (3 divisions).
3. At the Gated Amp Control screen, change the delay to 20 microseconds.

ATTENTION: The following adjustment must be made on the component side of the Quad 20/40/60 card (slot 10).

4. On the component side of the Quad 20/40/60 Card, slot 10, adjust R95 so the delayed FALS pulse has the same amplitude as the original non-delayed pulse (3 divisions).
5. Change the delay to 40 microseconds, and adjust R99 on the same card as above.
6. Change the delay to 60 microseconds, and adjust R103 as above.
7. Change the delay to 7 microseconds.

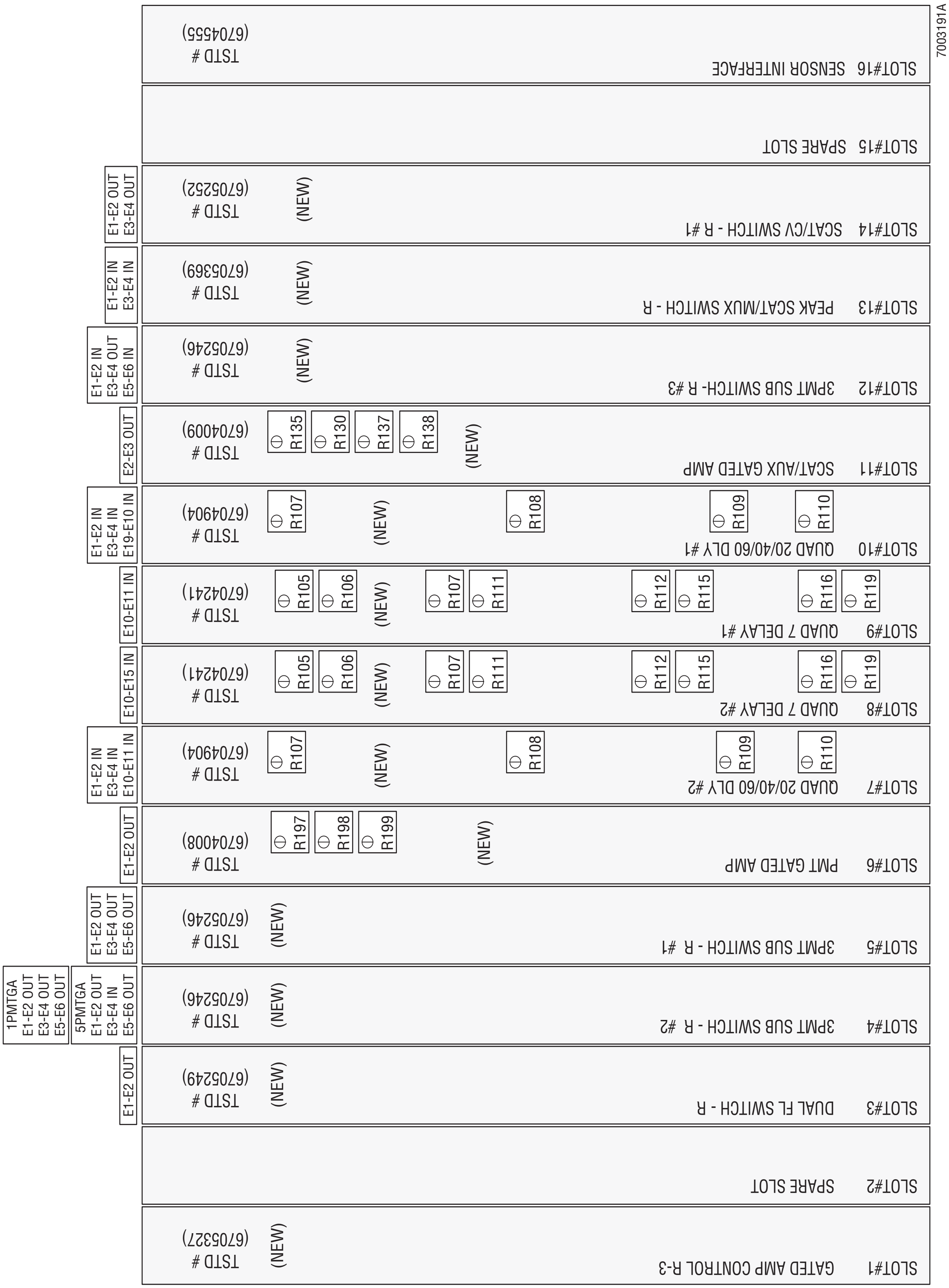


Figure 3.9-4 Gated Amplifier Assignment and Adjustment Potentiometer Locations

Table 3.9-3 PMT Adjustments

PMT	Slot	7 Microsecond Delay Adjustment	Slot	20 Microsecond Delay Adjustment	40 Microsecond Delay Adjustment	60 Microsecond Delay Adjustment
FALS	9	R105	10	R95	R99	R103
PMT1	9	R107	10	R96	R100	R104
AUX1	9	R112	10	R97	R101	R105
AUX2	9	R116	10	R98	R102	R106
PMT2	8	R105	7	R95	R99	R103
PMT3	8	R107	7	R96	R100	R104
PMT4	8	R112	7	R97	R101	R105
PMT5*	8	R116	7	R98	R102	R106

**Applies only if 5th PMT is installed.*

PMT1 Adjustment

See Table 3.9-3 and Figure 3.9-4 for additional information on adjustments.

1. At the Cytometer's Sort Screen:
   a. Turn the Strobe off.
   b. Reset the frequency to 32 kHz.
2. Open the detector compartment and remove the following filters:
   525 BP
   575 BP
   610 BP
   675 BP
   640 DL
3. At the Scope Screen, set the traces:
   a. Put PMT2 INT on Trace 1 (bottom).
   b. Put PMT1 INT on Trace 2 (top).
4. At the Option Screen, set Align LED to Pulsed, 100%, and 2 kHz.
5. At the Main Screen, adjust the pulses:
   a. Adjust PMT2 gain and HV gain for a three-division high pulse.
   b. Adjust the PMT1 gain and HV gain for a three-division high pulse.
6. At the Gated Amp Control Screen (Figure 3.9-5), select **FALS LWBM** and **PMT1 UPPBM**.
7. Ensure that the delay is set to 7 microseconds at the Gated Amp Control Screen.
8. Adjust R107 on the Quad 7 Microsecond Delay card (slot 9) for the same amplitude as in step 5.
9. At the Gated Amp Control Screen, change the delay to 20 microseconds.

ATTENTION: The following adjustment must be made on the component side of the Quad 20/40/60 Microsecond Delay card (slot 10).

10. Adjust R96 on the Quad 20/40/06 Microsecond Delay card, slot 10, as above.
11. Change the delay to 40 microseconds and repeat as above adjusting R100.
12. Change to 60 microseconds and adjust R104 as above.
13. Change the delay to 7 microseconds. Set to LWBW.
14. Press **SHEATH** at the Cytometer membrane panel.
15. At the Cytometer, go to the Options screen and set the **Align LED** to **Pulsed**, **100%**, and **2 kHz**.
16. Remove the 488 blocker, the 525 bandpass, the 575 bandpass and the 675 bandpass filters.

ATTENTION: Some instruments may not have a 625 DL but a 640 DL, which produces a ragged pulse on PMT 5. The 600 DL may be moved out under PMT4 to obtain signals for PMT 4 and PMT 5.

17. Verify that the 488 DL is under PMT1, 550 DL is under PMT2, 600 DL is under PMT3, and 625 DL is under PMT4.

Figure 3.9-5 Gated Amplifier Control Screen

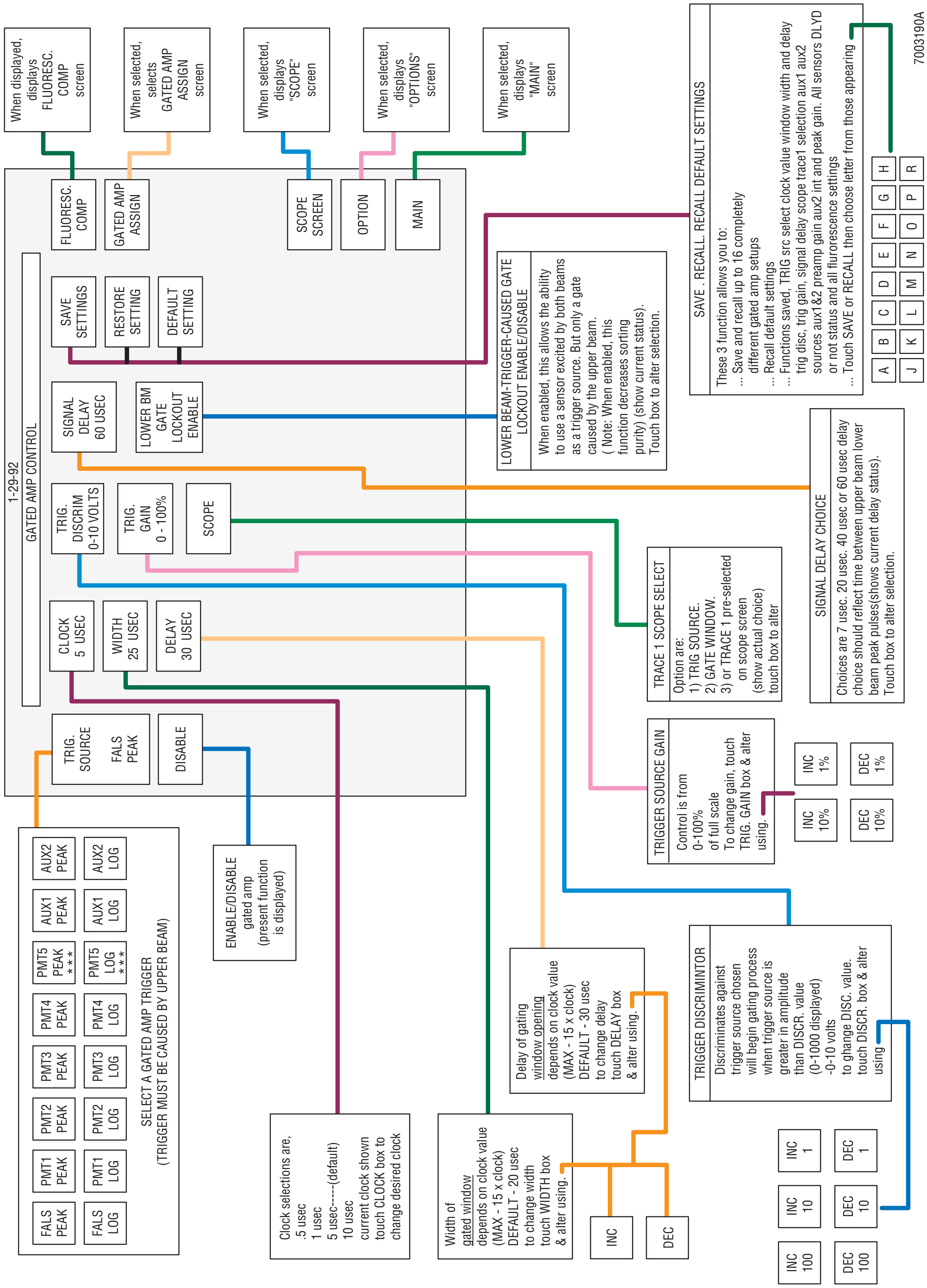
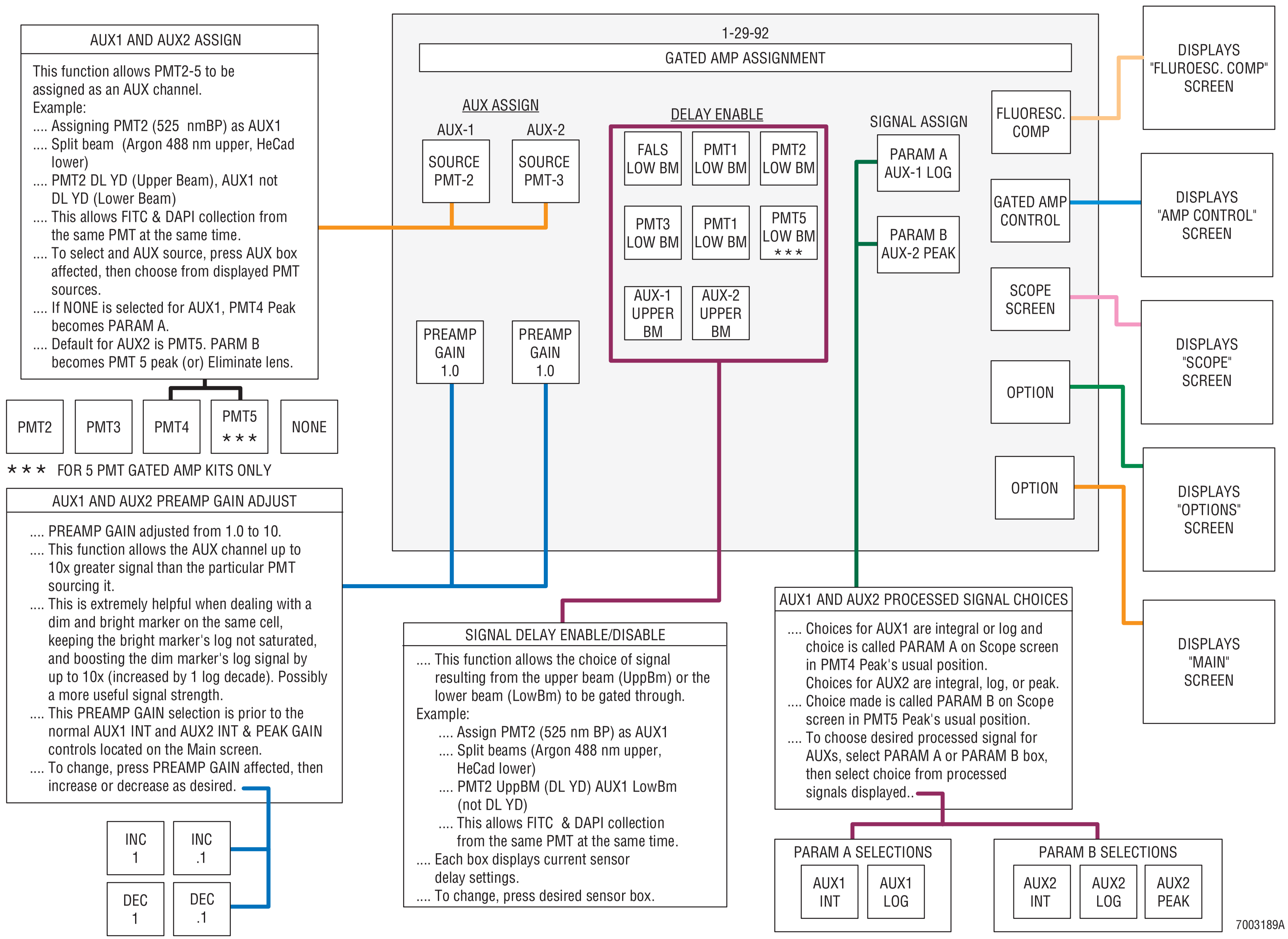


Figure 3.9-6 Gated Amp Assignment Screen



18. At the Cytometer's Scope screen:
    a. Put PMT1 INT on the bottom and PMT2 INT on the top.
    b. Adjust PMT2 HV and gain to get PMT2 to three divisions.
    c. Put PMT3 INT on the top trace and repeat as above, then do PMT5 if installed.
19. Put the mirror into the dichroic slot under PMT4 and adjust its voltage and gain as above. Go back to the scope screen and select a parameter a for the top trace.

AUX 1 Adjustment

See Table 3.9-3 for additional information on adjustments.

1. At the Cytometer's Scope screen:
   a. Set Trace 1 to PMT1
   b. Set Trace 2 to PMT2.
2. At the Cytometer's Gated Amp Assign screen, select:
   a. **AUX1 SOURCE** for PMT2
   b. **AUX2 SOURCE** for PMT2.
3. At the **PARM A** box, ensure **AUX1 INT** is selected, and at the **PARM B** box, ensure **AUX2 INT** is selected. Adjust the preamp gain so that pulse is three divisions tall.
4. Select **AUX1** for **UPBM**. Adjust R112 on the Quad 7 Microsecond Delay card, slot 9, so that the AUX1 pulse is the same amplitude as the non delayed original PARM A pulse (three divisions).
5. Go to the Gated Amp Control Screen and change the delay to 20 microseconds.

   Note: The following adjustments must be made on the component side of the Quad 20/40/60/ Microsecond Delay card, slot10.
6. Adjust R97 on the Quad 20/40/60/ Microsecond Delay card, slot 10, as above.
7. Change the delay to 40 microseconds and adjust R101 (slot 10) as above.
8. Change the delay to 60 microseconds and adjust R105 as above.

AUX 2 Adjustment

See Table 3.9-3 and Figure 3.9-4 for additional information on adjustments.

1. Change the delay to 7 microseconds.
2. At the Cytometer's Scope screen, select **PARM B** for the upper trace.
3. At the Cytometer's Gated Amp Assign screen, select **AUX2 UPBM** noting the amplitude as before.
4. Adjust R116 on the 7 Microsecond Delay card in slot 9, for the an amplitude of three divisions.
5. At the Cytometer's Gated Amp Control screen, select 20 microseconds delay.
6. Adjust R98 on the Quad 20/40/60/ Microsecond Delay card, slot 10, for an amplitude of three divisions.
7. Change the delay to 40 microseconds and adjust R102 for an amplitude of three divisions.

8. Change the delay to 60 microseconds and adjust R106 for an amplitude of three divisions.
9. At the Workstation, put the protocol in **RECEIVE CYTOSETTINGS AND SORT SETTINGS**.
10. Press F9 to receive the high voltages and gains.
11. Press F10 and change to the Protocol screen.
12. Change to **SEND CYTOSETTINGS AND SORT SETTINGS** and save the protocol as GATED AMP.
13. Turn off the unit.
14. Remove the Quad 20/40/60 Microsecond Delay card from the extender and install into slot 10.
15. Put the Quad 20/40/60 Microsecond Delay card, slot 7, onto the extender.
16. Turn on the instrument.
17. After Elite software is loaded, select **GATED AMP** protocol.
18. Press F9.
19. At the Cytometer's Options screen, set the **Align Led** to pulsed, **100%**, and 2kHz.
20. At the Cytometer's Scope screen, select **PMT1 INT** for the lower and **PMT2 INT** for the upper trace.

    Note: You should see two traces, each with a three division-high deflection body.
21. Press F10.

PMT 2 Adjustment

See Table 3.9-3 and Figure 3.9-4 for additional information on adjustments.

1. At the Cytometer's Gated Amp Control screen, select 7 microseconds delay. Verify that PMT2 INT pulse (upper trace) is 3 divisions tall.
2. Note the amplitude of upper trace, PMT2 INT. At the Gated Amp Assign screen, select **PMT2** as **UPBM**.
3. Adjust R105 on the Quad 7 Microsecond Delay card, slot 8, so the pulse is three divisions tall.
4. At the Cytometer's Gated Amp Control screen, set the delay to 20 microseconds.
5. Adjust R95 on the Quad 20/40/60 Microsecond Delay card so the pulse is three divisions tall.
6. Change the delay to 40 microseconds and adjust R99 so the pulse is three divisions tall.
7. Change the delay to 60 microseconds and adjust R103 as above for three divisions tall.
8. Set the delay to 7 microseconds.

PMT 3 Adjustment

See Table 3.9-3 and Figure 3.9-4 for additional information on adjustments.

1. At the Scope screen, select **PMT3 INT** as the top trace. Note the amplitude of PMT3 INT pulse (three-division tall trace)
2. At the Gated Amp Assign screen, select **PMT3 UPBM**.
3. Adjust R107 on the slot8 card so that the pulse is three divisions tall.

4. Change the delay to 20 microseconds and adjust R96 on the slot 7 card as above.
5. Change the delay to 40 microseconds and adjust R100 on the slot 7 card as above.
6. Change the delay to 60 microseconds and adjust R104 on the slot 7 card to get the amplitude as above.

PMT 4 and 5 Adjustment

See Table 3.9-3 and Figure 3.9-4 for additional information on adjustments.

1. Repeat the PMT 3 Adjustment procedure for PMT 4 and 5 (if installed). When finished, turn power off, remove the extender, and insert card into slot 7.
2. Remove the mirror from the PMT 3 slot and put it in the PMT 4 or PMT 5 slot as needed.
3. Offset adjustments for PMT 4 and PMT 5 as shown in in Table 3.9-4 and Figure 3.9-4.

Table 3.9-4 Offset Adjustments

PMT	Slot	Non-Delay Adjustment	Slot	7 Microsecond Delay Adjustment	Slot	60 Microsecond Delay Adjustment
FALS	11	R135	9	R106	10	R107
PMT1	11	R136	9	R111	10	R108
AUX1	11	R137	9	R115	10	R109
AUX2	11	R138	9	R119	10	R110
PMT2	6	R197	8	R106	7	R107
PMT3	6	R198	8	R111	7	R108
PMT4	6	R199	8	R115	7	R109
PMT5*	6	R200	8	R119	7	R110

**Applies only if PMT 5 is installed.*

4. Turn on the instrument, and after the Elite Software to loads, select the **GATED AMP** protocol.
5. At the Workstation's Parameter screen, select **FALS PARAMETER LOG**.
6. Press F9.
7. At the Sort screen, select **Strobe** and set **Frequency** to 2 kHz. Be sure the deflection body is all the way up and the FALS filter is removed.
8. At the Sort Gated Amp Control screen:
   a. Select **PMT1 PEAK** for the trigger source.
   b. Set the trigger discriminator to 100.
   c. Adjust the trigger gain to see the lower scope trace three divisions tall.
9. Put mirror under PMT1 and adjust HV to get 3 divisions.

10. At the Scope screen:
    a. Set lower scope trace to **TRIG**.
    b. Set upper scope trace to **FALS LOG**, and 10 microseconds/division.
11. At the Gated Amp Control screen:
    a. Raise the gain and voltage so PMT1 is displayed.
    b. Set the clock for 0.5 microseconds.
    c. Set the window width to 5.5 microseconds.
    d. Set the window delay to 5.5 microseconds and enable.
    e. Set the signal delay to 7 microseconds.
12. Change the display or scope trace to display window.
13. At the Gated Amp Assignment screen (Figure 3.9-6):
    a. Select **FALS LWBM**.
    b. Block the FALS sensor totally so it is totally covered and dark.
14. Connect the scope to the upper trace (UT) on the connector panel BNC next to the ac (under table top) left of the ac power outlets.

    Note: When forward scatter (FS) is blocked, no signal should be visible. If offset is wrong, you will see a signal either before the window (+offset) or after the window (-offset).
15. Adjust R135 (slot 11) Scat/Aux Gated Amp to minimize any pulse present on the upper trace during or after the window. View the scope output to see zero crossing.
16. Select **FALS UPBM** and adjust R106 slot 9 to minimize any pulse present on the upper trace during or after the window.
17. Repeat steps 13 through 14 with signals set one decade higher and center the adjustment potentiometer.
18. At the Gated Amp Control screen (Figure 3.9-5):
    a. Change the delay to 60 microseconds and change the clock to 5.
    b. Adjust R107 (slot 10) to minimize any pulse present on the upper trace during or after the window.
    c. Turn Strobe off.
19. At the Options screen, select **Align LED**, **Pulsed 100%**, and **Drive 2 kHz**.
20. Replace the mirror with the 488 DL filter.
21. Select **Display Trigger**.
    a. Change trigger source to **PMT4 PEAK**.
    b. Set the delay to 7 microseconds and change the clock to 0.5.
    c. Adjust PMT 4 HV for 3 divisions tall trigger pulse.

    Note: If PMT 5 is installed, change trigger source to **PMT5 PEAK**. Set the delay to 7 microseconds, and adjust PMT5 HV for 3 divisions tall TRIGGER.
22. At the Scope screen, put PMT1 LOG on the top pulse.

23. At the Gated Amp Assignment screen (Figure 3.9-6):
    a. Put PMT1 to **LWBM** and block the PMT 1 signal by putting one of the blanks supplied with the filter kit into the bandpass slot under it.
    b. Adjust R136 (slot 11) to minimize any pulse on the upper trace during or after the window.
    c. Select **PMT1 UPBM** and adjust R111 (slot 9) to minimize any pulse present on the upper trace during or after the window.

       Note: You may increase delay and open window up to the right of the signal.
24. At the Gated Amp Control screen (Figure 3.9-5):
    a. Set the delay to 60 microseconds and the clock to 5.
    b. Adjust R108 (slot 10) to minimize any pulse present on the upper trace.
    c. Disable the trigger gate
    d. Set the delay to 7 microseconds and the clock to 0.5.
25. At the Gated Amp Assign screen:
    a. Set AUX1 and AUX2 source to PMT2.
    b. Set PARM A to PMT2 LOG, PARM B to PMT2 LOG.
26. At the Scope screen, put PARM A on the top trace.
27. At the Gated Amp Control screen, enable GATED AMP.
28. At the Gated Amp Assign screen:
    a. Change AUX1 to the LWBM and block PMT2 signal by moving the blank into the bandpass slot.
    b. Adjust R137 (slot 11) to minimize any pulse present on the upper trace.
    c. Change AUX1 to UPBM.
    d. Adjust R115 (slot 9) to minimize any pulse present on the top trace.
29. At the Gated Amp Control screen:
    a. Change the delay to 60 microseconds and the clock to 5.
    b. Move the gate and windows out to right.
    c. Adjust R109 (slot 10) to minimize any pulse present.
    d. Change the delay to 7 microseconds and the clock to 0.5.
30. At the Gated Amp Assign screen, set AUX2 to the LWBM.
31. At the Scope screen:
    a. Put PARM B on the top trace.
    b. Adjust R138 (slot 11) to minimize.
32. At the Gated Amp Assign screen:
    a. Select AUX2 to UPBM.
    b. Adjust R119 (slot 9) to minimize.
33. At the Gated Amp Control screen:
    a. Change the delay to 60 microseconds and the clock to 5.
    b. Adjust R110 (slot 10) to minimize.

   c. Change the delay to 7 microseconds and the clock to 0.5.
   d. Change the trigger source to **PMT1 PEAK**.
   e. Verify that pulses are going into PMT 1, and arrange optical filters.
   f. Check Trig Signal of PMT1 Peak.
34. At the Scope screen, put PMT2 LOG on the upper trace.
35. At the Gated Amp Assignment screen:
    a. Ensure PMT2 is on the LWBM.
    b. Adjust R197 (slot 6) to minimize.
    c. Select **PMT2 UPBM**.
    d. Adjust R106 (slot 8) to minimize.
36. At the Gated Amp Control screen:
    a. Change the delay to 60 microseconds and the clock to 5.
    b. Adjust R107 (slot 7) to minimize.
    c. Change the delay to 7 microseconds, move the blank to the bandpass position under PMT3, and change the clock to 0.5.
37. At the Scope screen, put PMT3 LOG on the upper trace.
38. At the Gated Amp Assign screen:
    a. Ensure PMT3 is on the LWBM.
    b. Adjust R198 (slot 6) to minimize.
    c. Select **PMT3 UPBM**.
    d. Adjust R111 (slot 8) to minimize.
    e. Change the delay to 60 microseconds and the clock to 5.
    f. Adjust R108 (slot 7) to minimize.
    g. Change the delay to 7 microseconds, move the blank to the bandpass position under PMT4, and change the clock to 0.5.
39. Repeat steps 33 through 36 for PMT 4 and 5 (if installed) for the offset adjustments using Table 3.9-4. Use Align LED and 550 DCLP and mirror.
40. With **PMT1 PEAK** still selected as the trigger source, set the pulse delay for 7 microseconds and set the clock to 0.5 microseconds.
41. Adjust the window width for 6 microseconds.
42. Adjust the window delay for 6 microseconds.
43. Ensure the gated amp is disabled.
44. Set Signal delays for UPPBM.
45. At the Scope screen:
    a. Select trace 1 for TRIG.
    b. Select trace 2 for PMT2 LOG.
46. Ensure optical filters are arranged to allow signals in both PMTs used as trigger, and PMT being tested. Use a dichroic.
47. At the Main screen, adjust PMT2 HV for a 1 division tall log pulse.

48. At the Gated Amp Control screen, set **GATED AMP ENABLE**.

    Note: While monitoring PMT2 on trace 2, the amplitude should remain 1 division tall and should not increase or decrease when the gated amp is enabled or disabled.

    If the signal changes in amplitude, adjust R106 in slot 8 (Quad 7 Microsecond Delay card) until no change occurs.

49. See Table 3.9-4 for Sensor VS Offset pots to adjust for offsets in the 7 Microsecond Delay card. Do this for PMT1, PMT2, PMT3, PMT4, PMT5 (if installed), AUX1 and AUX2.

    Note: When setting the PMT to be tested for 1 division tall log on trace 2 on the scope, disable the gated amp.

    Note: Use a sensor not being tested for the gated amp trigger source so amplitude will be large enough to start gating process.

50. At the Gated Amp Control screen, select the following:

PMT1 PEAK	=	TRIGGER SOURCE
CLOCK	=	5 msec
WINDOW WIDTH	=	20 msec
WINDOW DELAY	=	50 msec
SIGNAL DELAY	=	60 msec.

    Note: **WINDOW DELAY** can be adjusted as necessary (30,45,50,etc) to capture complete pulse.

51. Repeat step 50 for PMT1, PMT2, PMT3, PMT4, PMT5, AUX1 & AUX2 for 60 microsecond pulse delay using Table 3.9-4 for pot for signals needing adjustment.

52. Adjust the offsets for the 7 microseconds delay using the same procedure in step 50. The pots will be different for each card. See Table 3.9-4.

53. Do the correct laser alignment.

Laser Alignment With Gated Amplifier

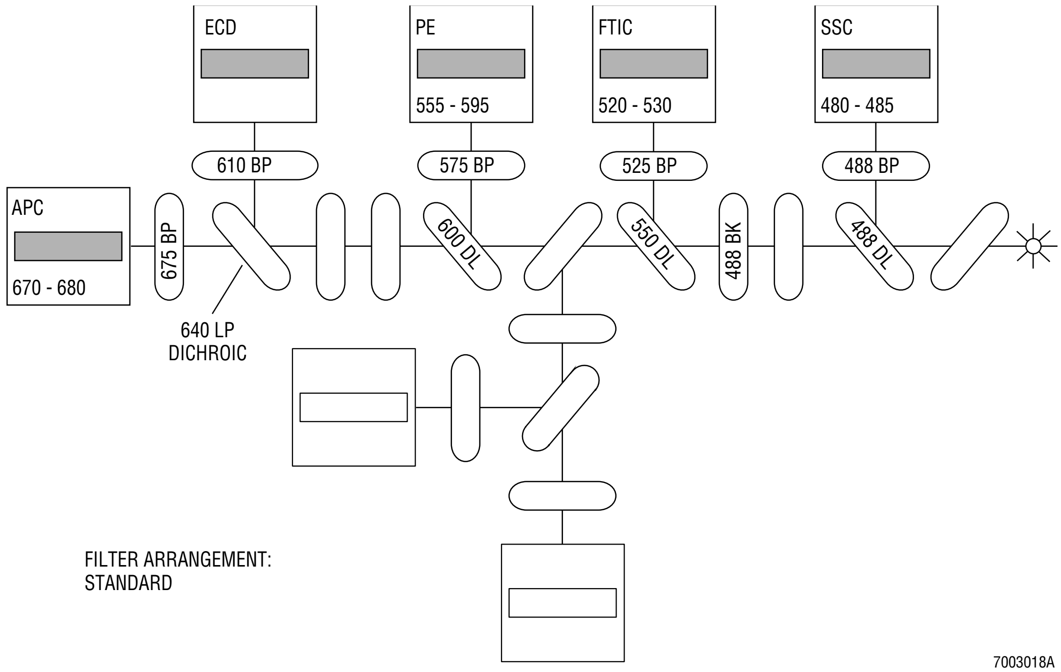
Laser Configuration

One Innova 305 laser is set to the UV mode and placed in the rear-most position on the optical bench and parallel to the PMT block. A beam expander/contractor is used to insure beam diameter is the same for both lasers.

A second Innova 305 laser is set to the 457-nm line and placed parallel to the first Innova 305 laser. If necessary, it is possible to position these two lasers close enough together so allow both the Argon air-cooled and the HeNe lasers to be positioned also.

Figure 3.9-7 shows a standard laser configuration.

Figure 3.9-7 Standard Laser Configuration



Laser Alignment

1. Install the front (457-nm) laser using both targets with no optics. The beam should go cleanly through both targets to ensure a beam parallel to the optical bench.
   a. Place a piece of paper (or some non-flammable material) on the wall opposite the laser to establish a wall target.
   b. For Elite configured units, once the laser is level, lower the laser 1/16 inch by marking a spot below each target and checking them one at a time (since the beam will no longer go through the target holes).
   c. For Analyzer configured units, once the laser is level, raise the laser 1/16 inch by marking a spot below each target and checking them one at a time (since the beam will no longer go through the target holes).
2. Install the back (UV) laser using the targets. Do not move the laser.
3. Measure the spots on the wall between both beams to ensure the distance apart is the same as the laser beam exit point on each laser.

   Note: Both lasers should be exactly parallel, but separated by 1/16 inch.
4. Install the UV dichroic mirror and beam expander. Target beam through to target the FLS position. Use all four targets.
5. Install the 457 dichroic mirror and repeat step 4 with the front laser. Do not worry about the beam displacement at this point; you will use the for pulses for fine tuning.
6. Run beads and establish peak signal pulses. Adjust beams to see a 40 microsecond delay. Pay special attention to focus. You will find that you must compromise the focus to get the best pattern for both beams, if the beam heights change greatly during focus.

7. Use targets to block each beam in turn to make sure you know which pulse is associated with which beam.
8. Delay appropriate signals, establish the window and enable the gated amplifier.
9. When running beads, the UV power must be high because the beads fluoresce brighter in the 457 nm region. When running chromosomes, the Hoechst signal is usually much brighter than the Mithromycin, so the 457 laser can be run almost at full power and the UV laser must be reduced. You can then adjust PMT voltages, and so forth, for fine tuning.

Calibration

1. Align both lasers with tall targets so both are parallel to each other and the optical table. Ensure the beam width at the beam origin and at the wall, at least 3 feet away, is the same.
   - For Elite instruments, the front laser (upper beam at flow cell) should be 1/32 in. lower. This means the beam will clip the bottom hole of both targets.
   - For Elite Analyzer instruments, the front laser (upper beam at flow cell) should be 1/32 in. higher. This means the beam will clip the bottom hole of both targets.
2. Install small targets on optical plate. (Remove FALS detector and laser shutter.)
3. With the lens block and flow cell moved to their highest position of travel, aim front laser (delayed signals) so that back target just clips beam on bottom of hole and front target just clips the beam on the top of the hole. (Use 488 dichroic mirror adjustments to target.)
4. Aim the rear laser (delayed signals) directly through both targets using UV dichroic mirror adjustments.
5. Install the beam reducer/expander and re-target the rear laser beam using the beam reducer/expander position adjustments. Ensure that beam passes through center of telescope lens.
6. Lower beam shaper lens into the beam path and recheck the beams, especially for side to side position. The beam shape on the back laser target will be elliptical, not round. Use beam shaper Y- and Z-axis adjustments to target beams as in steps 4 and 5.
7. Lower the flow cell into the beam path and reposition the flow cell mounting plate as necessary to ensure sample stream is intersected by beams. DO NOT adjust beam shaper Z-axis to do this.
8. Remove targets and reinstall FALS detector and laser shutter.
9. Shut off back laser. (Close aperture while in current mode.)
10. Run beads and establish pulses for both FALS and fluorescence. Make all usual adjustments to ensure good CVs and sensitivity.
11. Turn on beam from back laser. Adjust power on lasers so pulses are nearly equal. (With DNA check beads this means about 10X power for UV than for visible wavelengths.) Optimize pulses using telescope controls.
12. Adjust peak signals form both lasers so they are 40 microseconds apart for 100 µm quartz and 7 microseconds apart for jet-in-air.
13. Cycle through several adjustments of all alignment controls to obtain good pulses from both beams.

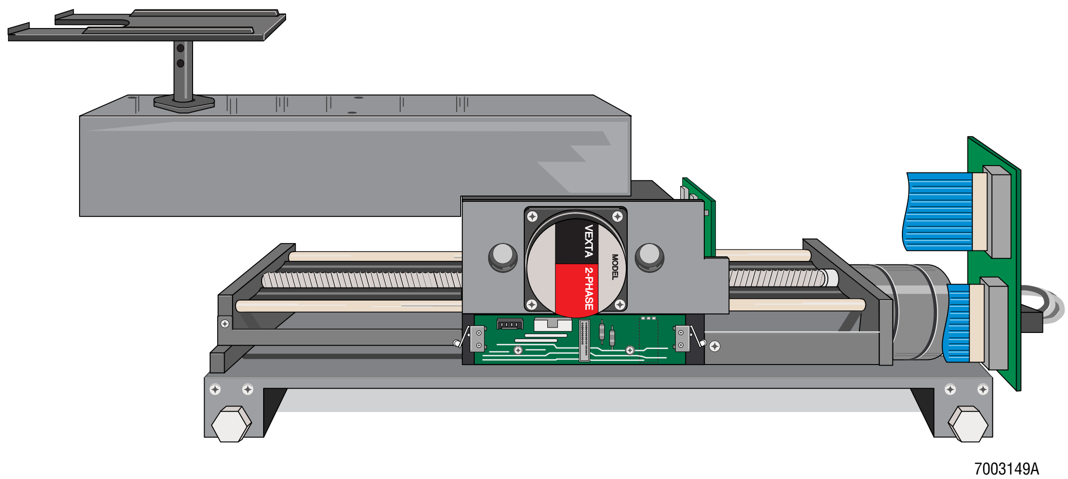
14. If the peak signal pulse width is not very similar for signals from both lasers, minimize the pulse width for the front laser by adjusting the beam shaper focus, and minimize the rear laser peak signal pulse width by adjusting the beam reducer/expander. You may have to constantly reposition the back beam with the telescope control at the same time.
15. Once both beams look good, go to the Gated Amp Control screen and set the clock delay and width. Set delay to 40 microseconds for 100 µm quartz and 7 microseconds for jet-in-air.
16. Set source to some signal associated with the front laser beam.
17. Delay all signals associated with this beam.
18. Turn on gate window display and adjust accordingly so that the entire peak signal pulse width is enclosed by the window.

3.10 AUTOCLONE SORTING OPTION INSTALLATION

Purpose

Use this procedure to install the Autoclone Sorting Option mechanism (Figure 3.10-1).

Figure 3.10-1 Autoclone Sorting Option Mechanism



Tools/Supplies Needed

- ❑ Autoclone Sorting Option Kit, PN 6912869-6
- ❑ Connector mounting block, PN 1016713-2
- ❑ Cable assembly, PN 6028268-4
- ❑ Phillips-head screw, PN 2804039-3
- ❑ Foam for panel, PN PN 3203037-8
- ❑ Solenoid valves, PN 6232096-6
- ❑ Fitting, PN 6232086-9, with seal, PN 2523062-1, for manifold
- ❑ Miniature fitting, PN 6232413-9, for solenoid valve position 13
- ❑ Actuator, PN 6211015-5, for solenoid valve position 13
- ❑ Pinch valve, PN 6855763-1, for solenoid valve position 13
- ❑ Hex nut, PN 2822033-2, for solenoid valve position 13
- ❑ Fittings, PN 6232104-1, with nuts, PN 2822072-3, for holes FF39, FF33, and FF42 on mounting plate
- ❑ Fitting, PN 6232417-1, with nut, PN 2822016-2, for hold FF38 on mounting plate
- ❑ 7.5 in. (19 cm) tubing, PN 3203015-7
- ❑ Tubing, PN PN 3213063-1, for manifold position 14
- ❑ Fitting, PN 6232109-1, for end of tubing
- ❑ Tubing, 9 in. (23 cm), PN 6303015-5
- ❑ I-beam tubing, PN 3213136-1

- ❑ Tubing, longer piece, PN 3202039-9
- ❑ T-fitting, PN 6216129-9
- ❑ Tubing, 1 in. (3 cm) for end of new T-fitting, (PN 3202039-9)
- ❑ Wire tie mount, PN 6011017-4
- ❑ Wire tie, PN 6011001-8
- ❑ Double-sided tape, PN 8023259-4
- ❑ Metal strip, PN 1021020-8
- ❑ Autoclone Sorting Option mechanism screws, PN 2839073, and washers, PN 2827135-2
- ❑ Deflection body assembly, PN 6704311-1
- ❑ Gloves
- ❑ Software version 4.01 or above is required

Electrical and Mechanical Inspection

1. Verify that the 256K Memory card (or Cytometer CPU card) has been installed.
2. Verify that the instrument has been upgraded to have the thin insertion body flow cell and tip with the 3000 Vdc modules.
3. Verify that there are no broken components on the Autoclone Sorting Option Interconnect and Autoclone Sorting Option Position cards.
4. Verify that there are no loose or damaged mechanical components.
5. Verify that Bio-Hazard Upgrade Kit has been installed.
6. Verify that the Elite system sorts correctly. Make corrections before proceeding.

Preliminary Procedure

1. Turn power switch OFF.
2. Remove the front right panel (Data Acquisition Card cage cover).
3. Remove the Sort Oscillator card.
4. Replace R7 with a 4.99 kOhm resistor.
5. Replace R12 with a 10 kOhm resistor.
6. Install the Sort Oscillator card.
7. Remove the deflection body.
8. Remove the sample collection drawer.
9. Remove the existing 1/4-turn brackets from frame where sample collection drawer was fastened.
10. Remove the back panel to provide access to Motor Controller card cage.
11. Open the panel on front of the Cytometer, left of the sample delivery door.
12. Remove the optical filters from optical block.
13. Remove the optical block cover.

14. If the lip on the laser interlock assembly, where the assembly fastens to optical table near the flow cell area, does not face the front of the Cytometer and if the bolt heads are not exposed:
    a. Remove the laser interlock assembly.
    b. Remove the left side panel from laser interlock assembly.
    c. Transfer components from laser interlock assembly panel just removed to new laser interlock assembly panel.
    d. Place new foam on new panel using old panel for reference.
    e. Install laser interlock assembly on optical table.
15. Remove the Motor Controller card from the lower slot.
16. Install the connector mounting block over 44 pin card edge connector of the cable assembly.
    a. Make sure the stubs with the threads are towards the side of the wires.
    b. Work the connector into the Cytometer from where the sample collection drawer was to the rear of the Motor Controller card cage.
17. Place the 44 pin connector into the empty slot above the Motor Controller card slot, and verify that the letters side is up.
18. Fasten the screw to the threads in the connector mounting block:
    a. Tape the screw to a Phillips-head screwdriver, or use a crew-retaining Phillips-head screwdriver.
    b. Bring the screw to the connector inside the Motor Controller card cage and fasten the screw to the threads in the connector mounting block.
19. Install the Motor Controller card in the bottom slot.
20. If the instrument is not Autoclone Sorting Option-Ready from the factory, do Upgrading the Instrument to be Autoclone Sorting Option-Ready

Upgrading the Instrument to be Autoclone Sorting Option-Ready

Do this procedure if the instrument is not Autoclone Sorting Option-ready

1. Install the Autoclone Sorting Option card into the empty slot above the Motor Controller card.
2. Open the pneumatic housing door.
3. Remove the screw-plugs from the 13th and 14th valve positions (second and third positions up from the bottom of the pneumatic manifold).
4. Disassemble the new solenoid valves noting the position of the internal assemblies.
5. Install solenoid valve bottoms at positions 13 and 14 and assemble the solenoid valves.
6. Fasten terminals with WM 342 and WM 343 on the adjoining cable assembly to the solenoid valve at position 14.
7. Fasten terminals with WM 340 and WM 341 on adjoining cable assembly to the solenoid valve at position 13.
8. Install a fittings with a seal on the manifold at a right angle to the solenoid valve as position 14.

9. Install miniature fitting, actuator, pinch valve, and hex nut on manifold at right angle to solenoid valve at position 13.
10. Install fittings with nuts on holes marked FF39, FF33, FF42 on mounting plate.
11. Install a fitting with a nut on the hole marked FF38 on the mounting plate.
12. Route the 7.5 in. piece of tubing through the hole nearest the manifold to the new pinch valve at position 13.
13. Connect the tubing to FF38 and FF39 to pinch valve side of the bracket.
14. Connect tubing to the fitting at the manifold position 14.
15. Route the other end of the tubing through the hole on the mounting plate towards the backside of the mounting plate.
16. Pass tubing through the opening on the top of the pneumatic housing. Pass tubing through the frame (to be connected later).
17. Connect the remaining piece of tubing to FF38.
18. Connect the tubing:
    a. Route the other end of tubing through the opening on top of the pneumatic housing.
    b. Pass tubing through the frame.
    c. Connect fitting to the end of the tubing.
    d. Connect 9 inches of the tubing and leave until later.
19. Install the I-beam tubing through the pinch valve at position 13 to fittings FF33 and FF42 on the pinch valve side of the bracket.
20. Remove the waste tubing from FF15 on the rear of solenoid side of the bracket.
21. Remove the brass T-fitting.
22. Connect the tubing from the top of the T-fitting coming from FF21 to FF15.

    Note: A longer piece of tubing will be needed.
23. Connect the tubing that was going to top of the removed T-fitting to FF33.

    Note: A longer piece of tubing will be needed. Do not remove check valve.
24. Remove tubing from the bottom of the T-fitting coming from FF36.
25. Add 1 inch (3 cm) of the tubing from the bottom of the T-fitting coming from FF36.
26. Install the top of the new T-fitting to the 1-inch piece of tubing.
27. Connect the tubing that you removed in step 24 to the bottom of the new t-fitting.
28. Install the 1-inch (3 cm) piece of the tubing on the remaining end of the new T-fitting.
29. Install the T-fitting on the 1-inch (3 cm) piece of tubing just installed.
30. Connect the remaining ends of T-fitting to FF39 and FF42 with tubing.
31. Remove the two screws holding the terminal block assembly mounted to the frame.
32. Pull terminal block out to where you can access the terminals.
33. Connect the spade lugs from the cable assembly to the terminals according to Table 3.10-1.

Table 3.10-1 Spade Lug Connections

From Wire Marker (WM) Number	To Terminal Block Label
None	STROBE -
None	STROBE +
None	DEFL +
None	DEFL -
None	+12V
None	+15V
433 (Orange)	+15V
432 (Wht/Blk)	ANLG GND
None	ANLG GND
434 (Green)	-15V
None	-15V
438 (Black)	DGT GND
None	DGT GND
None	DGT GND
None	+5V
None	+5V
437 (Yellow)	+5V
435 (Brown)	+24V
None	+24V
None	+24V
436 (Grn/Yel)	CHAS GND
None	AC NEUT
None	AC NEUT
None	AC HOT
None	AC HOT

34. If wires from the deflection body holder are running down in front of the optical plate:
    a. Taking care to note their location, remove the wires from the terminal block assembly.
    b. Route the wires horizontally along the optical plate toward the back of the unit.
    c. Wrap the wires along the back and insert wires down through the hole on the PMT plate.
    d. Reconnect wires to terminal block assembly in their original location.

35. Reroute any other cables or tubing coming up along the front of the optical table through this same hole on the PMT plate.
36. Place a wire tie mount on the front of the optical plate, taking care it does not interfere with anything.
37. Use a wire tie to clamp all the cables and tubing to the wire tie mount.
38. Replace the terminal block assembly, and fasten with screws from step 31.
39. Place the Autoclone Sorting Option mechanism under the optical table with 37 pin D connector to rear of Cytometer, directly behind the sample collection area.
40. Check the level of the Autoclone Sorting Option to Cytometer optical table.
    - If the Autoclone Sorting Option is not level, use double-sided tape to fasten the metal strip to the top of the inner frame rail.
    - If Autoclone Sorting Option arm is level, but there is no clearance between the bottom of the table and the top of the arm, then adjust optical table for about 1/8 in. clearance.
41. Loosely fasten the Autoclone Sorting Option mechanism with screws and washers to the frame.

    Note: Be sure to leave access to fan assembly cable.
42. Connect 37-pin D connector of cable assembly to Autoclone Sorting Option Interconnect card.

Calibration Procedure

1. Turn power ON and install new software. See Heading 4.9, SOFTWARE INSTALLATION.
2. At the Cytometer screen, select **Options ▸▸ Autoclone Sorting Option Control ▸▸ Adjust Screen ▸▸ Calibrate ▸▸ Load**.
3. Verify that after certain movements, the Autoclone Sorting Option stops at the load position.
4. Place the tray holder into arm with notch toward the front and the tray parallel to optical table and secure with set screw.
5. Place 96-well tray into holder with letters to front.
6. Select **Load** at the Cytometer screen. Verify that the arm moves and that when it stops, the sheath is in center of well A12. If not, adjust screws securing the Autoclone Sorting Option mechanism.

   Note: Press **SHEATH** to determine location of stream, then **VACUUM** to stop flow. Do not overflow the sample tray.
7. Select the **A1** position on the screen to move tray to selected location. Adjust the unit so that the sheath is in center of well A1.
8. Repeat steps 6 and 7 as necessary to align tray. Select position H12 and check stream position. Repeat steps 6 through 8 as needed.

   Note: You may need to readjust the tray-to-arm parallelity for optimal alignment.
9. Remove the tray and tray holder, and select Retract on the screen. Observe that the arm moves all the way back.
10. Connect the fan assembly cable to the fan on the new outside cover assembly.

11. Connect the hard tubing coming through the opening on the frame to the fitting on the cylinder.
12. Connect the soft tubing to the fitting on the beak.
13. Install the outside cover assembly, ensuring that tubing and cables are out of the way of the tray.
14. Repeat steps 4 through 9 and ensure that neither the arm nor the tray assembly hits the outside cover. If it does, check the cover for proper installation.
15. Install the Autoclone Sorting Option cover into sample collection area, securing with the knob to front of the Autoclone Sorting Option arm.
16. Remove the beak assembly from the old sample collection drawer and install into bezel cover assembly.
17. Transfer sample collection drawer accessories from old cover to new cover.
18. Install the new deflection body assembly.
19. Reinstall and secure all panels and covers removed in this procedure.

Adjusting Autoclone Sorting Option Speed

1. At the Cytometer screen, select **Options** ▸▸ **Autoclone Sorting Option** ▸▸ **Control Adjust Screen**.
2. Adjust the Autoclone Sorting Option's speed:
   - Use the XY adjustment to change the speed that the mechanism moves front to back and left to right.
   - Use [↑] and [↓] to increase or decrease the speed as needed.
3. Use the Rotation adjustment to change the speed of rotation of the plate holder.
4. Press **Calibrate** and observe the mechanism closely after any speed change.

   Note: Speed adjustments ensure that the mechanism operates smoothly without binding. Operation at too high a speed results in faults during the Calibration cycle. Too high a rotational speed results in the plate holder over rotating causing a fault condition.
5. The new speed values are retained by the system until Cytometer software is reloaded.
6. Note the numeric speed values for future reference.

Testing the Autoclone Sorting Option Mechanism

You can perform two tests to test the mechanical and electronic systems:

- In the first test, the mechanism simply steps through each well position. This provides a test of the movement mechanism.
- The second test generates sort pulses based on histograms acquired using FL test as the signal source. This test provides further confidence in sorting electronics operation.

See Chapter 6 of the Options manual for additional information on adjustments.

Prepare the System

1. At the Cytometer screen:
   a. Select **Options**.
   b. Set% **DRIVE** to 100

   c. Set **FREQ** to 0.25 kHz.

2. Remove the bandpass filters from under the PMTs; leave the dichroic filters in place.
3. At the Workstation:
   a. Create a protocol to acquire PMT1 (90 degree light scatter) and PMT2 as single parameter histograms.
   b. Set the **Discriminator** for PMT1 to 100; set all others to OFF.
4. Acquire the FL test pulses and adjust gains and high voltages as needed to obtain histograms.
5. At the Workstation Sort screen
   a. Create a region to include the population acquired from PMT2.
   b. Name the region **Test**.
6. Press Esc to return to the Sort screen.
7. Use the mouse to select the **Test** region in the region list box. When selected, the Test region turns white.
8. Move the mouse cursor to the box at the lower center of the screen and click on one of the ID numbers. Enter the test name Test when prompted.

   Note: The name and sort equation appear next to the ID number selected. Do not enter any equations into the **SORT RIGHT** or **SORT LEFT** boxes.
9. At the Cytometer's Autoclone Sorting Option screen, select:
   a. **Plate**, and choose the 96-well plate.
   b. **Orifice Diameter** and enter flow cell tip being used (100).
   c. **Well Timeout** and enter 1 second.
   d. **Test** equation.
   e. **Fill Plate** and select **Fill Plate** again.
10. Enter a count at the prompt (10).
11. When prompted for Description, press Enter. The plate displayed on the screen fills with the ID number of the selected equation.

First Test

1. Set **FL Test% DRIVE** to 0
2. Select **Activate**. The Autoclone Sorting Option steps through each well on the plate and returns to the Load position when complete.

   Note: The wells turn red since no sort pulses are present.

Second Test

1. Set **FL Test% DRIVE** to 100%
2. Start acquisition and ensure a population builds up within the sort region, Test, created earlier.
3. Select **Activate** and observe that the Autoclone Sorting Option steps through the wells of the plate.

   Note: The wells turn green indicating a successful sort for each well.

Stream and Waste Catcher Adjustment

When sorting with the Autoclone Sorting Option, the Deflection Gain and the Stream control (located above the fluidic controls) are adjusted such that the waste stream is constantly deflected to the right into the waste catcher and the sort steam drops straight down on the collection plate.

The waste catcher has two positions. In the extended position, it catches the waste stream and the sort stream. When retracted, it catches only the waste stream.

1. Turn on Sheath and use **Sort Test** to setup sort streams. Verify stable droplets and sidestreams.
2. Turn the Stream knob until the left sort stream is flowing straight down.
3. Switch the deflection high voltage on and off to check the stream position. With high voltage on, the left stream should look the same as the sheath stream does when high voltage is off.

   Note: You can control the waste catcher from the Cytometer Control Valves screen.

   - With Autoclone Sorting Option waste **OFF**, the waste catcher is retracted.
   - With Autoclone Sorting Option waste **ON**, the waste catcher is extended.
4. With the waste catcher retracted, adjust the **Deflection Gain** and move the waste catcher from left to right until the left side stream (the one flowing straight down) does not hit the waste catcher and the waste stream enters the waste catcher cleanly.
5. Verify that the waste catcher is correctly positioned so the extended and retracted positions function correctly.

   Note: Loosen the clamp holding the air cylinder which moves the waste catcher and adjust as needed.

Operational Test

Many factors affect the outcome of this test; the presence of liquid at all well locations and particles in some wells indicates Autoclone Sorting Option function. Actual results depend on the condition and adjustment of the host instrument.

1. Prepare a sample of fluorospheres and place a sort region around the fluorospheres.
2. Configure the system to sort 1 particle into each well of a 96 well plate.
3. Place the cover of the well-plate into the Autoclone Sorting Option and sort.
4. Check the plate cover with a microscope to confirm sorting.

3.11 5TH PMT KIT WITH SWITCHABLE AMPS INSTALLATION PROCEDURE

Purpose

Do this procedure to install PMT 5 on instruments that have switchable amplifiers.

Tools/Supplies Needed

- ❑ Mod Kit, 5 PMT Switch Amps, PN 6913056-9
- ❑ Elite - minimum software version 4.01
- ❑ Analyzer - minimum software version 4.01
- ❑ Bertan Supply No. 5 card, PN 6804637-8
- ❑ Analyzer HV DAC card, PN 6703941-6
- ❑ HV coax cable, PN 6027024-4
- ❑ PMT, PN 6856833-1
- ❑ 6-32 x 1.5 in. thumbscrew, PN 2815003-2
- ❑ PMT 4 cable, PN 6028205-6
- ❑ PMT signal coax cable, PN 6028205-6
- ❑ 3 PMT Sub Amp Amp SW-R2 card, PN 6705246-3
- ❑ BNC T-connectors (2), PN 2121537-6
- ❑ BNC bullet coax cables, short, (4), PN 6027482-7
- ❑ 27 in. (69 cm) bullet-bullet coax cable (3), PN 6027803-2

Hardware Configuration and Installation

1. Turn power OFF.
2. Remove the top cover, rear panel to expose the switcher and front right panel to expose the data acquisition and gated amp card cages.
3. Open the rear left door to expose the Bertan card cages.
4. Remove the lower Bertan card cage card retainer.
5. Install new Bertan Supply No. 5 card in fourth card slot from the left (including the slot for the HV DAC card) in the lower Bertan card cage.
6. Configure new Analyzer HV DAC card as HV DAC 2 by jumpering:
   - E5 - E9
   - E6 - E10
   - E7 - E11
   - E8 - E12
7. Install Analyzer HV DAC card in far left slot of the lower Bertan card cage, with the component side to the right, and the IC part of the card facing outwards. Install the ribbon cable connector with pin 1 towards the top. (Markings inside connector).
8. Install lower Bertan card cage card retainer.
9. Fasten new Bertan 5 power supply HV cable to bracket PMT 5 HV connector position.

10. Route the new HV coax cable the same as the other four HV coax cables and connect one end to Bertan card cage bracket PMT 5 connector position.

    Note: The new PMT becomes PMT 4, and the previous PMT 4 becomes PMT 5.

11. Install the new PMT in fourth PMT position from right facing the instrument. Lock in position with a 6-32 X 1/2 in. thumbscrew.
    a. Connect DC Power connector to an open position on the PMT DC Power Distribution card under the HeNe Laser.
    b. Connect BNC male end of new cable to new PMT Signal connector.
        1) Label each end of the cable PMT 4.
        2) Route cable with previously routed PMT signal cables.
    c. Connect existing PMT 4 HV cable to newly installed PMT 4 HV connector.
    d. Connect newly installed PMT 5 HV cable to old PMT 4 HV connector (new PMT 5). Relabel old PMT 4 signal BNC connector as PMT 5.
    e. Remove PMT 3 Signal coax cable from instrument and replace it with new PMT signal coax cable. Label both ends PMT 3 and route with existing signal cables.
12. Configure 3 PMT Sub Amp Amp SW-R2 card as follows:

    Note: Solder pin 8 and 9 of U11 Sub SW-R 1 card and 2 card for Software Version 3.1 and below.

    - Jumper E3-E4 In
    - Jumper E1-E2, E5-E6 Out
13. Install 3 PMT Sub Amp Amp SW-R2 card in the slot to the left of the existing 3 PMT Sub Amp Amp 1 card (Quad 20/40/60 Microsecond Delay card slot).

Cabling Configuration and Installation

1. Remove the following bullet connectors from 3 PMT Sub Amp SW-R card 1.
    - FL3 Int Out (J8). Relabel as PMT 3 Int
    - FL3 Peak Out (J6). Relabel as PMT 3 Pk
    - FL3 Log Out (J7). Relabel as PMT 3 Log.
2. Remove the following bullet connectors from the Dual FL SW-R card:
    - FL2 Int Out (J7). Relabel as PMT 4 Int
    - FL2 Peak Out (J6). Relabel as PMT 4 Pk
    - FL2 Log Out (J8). Relabel as PMT 4 Log.
3. Remove bullet connectors from 3 PMT Sub Amp SW-R 1 FL2 out (J5). Relabel cable ends as PMT 4 Out and PMT 4 In and connect the cables:.
    a. PMT 4 Out end to 3 PMT Sub Amp SW-R 2 FL2 out (J5)
    b. PMT 4 In end to Dual FL SW-R 2 FL2 In (J5)
4. Connect a BNC T-connector to PMT 3 Signal coax
5. Connect a BNC T-connector to PMT 4 Signal coax
6. Connect two short BNC bullet coax cables to PMT 3 BNC T-connector and label bullet ends as PMT 3.

7. Remove signal coax from 3 PMT Sub Amp SW-R 1 FL3 in (J3). Relabel as PMT 5, and connect:
   - One PMT 3 signal coax to 3 PMT Sub Amp SW-R 2 FL1 (J1).
   - The other PMT 3 signal coax to 3 PMT Sub Amp Sw 1 FL3 (J3).
8. Remove coax from FL2 in (J2) on 3 PMT Sub Amp SW-R 1 card. Disconnect BNC from PMT 3 and remove cable from instrument.
9. Connect two short BNC bullet coax cables to the PMT 4 BNC T-connector. Label the bullet ends as PMT 4 and connect:
   a. One PMT 4 signal coax to 3 PMT Sub Amp SW-R1 FL2 in (J2).
   b. Second PMT 4 signal coax to 3 PMT Sub Amp SW-R2 FL in (J2).
10. Connect PMT 5 signal coax to 3 PMT Sub Amp SW-R2 FL3 in (J3).
11. Connect PMT 3 Int coax cable to 3 PMT Sub Amp SW-R1 (FL3 Int Out) (J8).
12. Connect PMT 3PK coax to 3 PMT Sub Amp SW-R1 (FL3 Peak Out) (J6).
13. Connect PMT 3 log coax to 3 PMT Sub Amp SW-R1 (FL3 Log Out) (J7).
14. Connect (PMT 4 Int) signal to Dual FL SW-R FL2 Int Out (J7).
15. Connect (PMT 4 Pk) signal coax to Dual FL SW-R FL2 Pk Out (J6).
16. Connect (PMT 4 Log) signal coax Dual FL SW-R FL2 Log Out (J8).
17. Label one end of a 27 in. bullet-bullet coax PMT 5 Int. Label the other end MUX J22.
18. Label one end of a 27 in. bullet-bullet coax PMT 5 Log. Label the other end MUX J23.
19. Label one end of a 27 in. bullet-bullet coax PMT 5 Peak. Label the other end MUX J24.
20. Connect the PMT 5 Int signal coax to 3 PMT Sub Amp SW-R2 FL3 Int Out (J8).
21. Connect the PMT 5 Log signal coax to 3 PMT Sub Amp SW-R2 FL3 Log Out (J7).
22. Connect the PMT 5 Peak signal coax to 3 PMT Sub Amp SW-R2 FL 3 Peak Out (J6).
23. Connect the MUX J22 end of the coax to Mux and Scope (J22).
24. Connect the MUX J23 end of coax to Mux and Scope (J23).
25. Connect the MUX J24 end of coax o Mux and Scope (J24).
26. Relabel and install the following 27 in. cables as indicated in Table 3.11-1.

Table 3.11-1 Card and Cable Connections

From	To
3 Sub 1 (FL3 Int Out) (J8)	Mux and Scope Int (J16)
3 Sub 1 (FL3 Log Out) (J7)	Mux and Scope Int (J17)
3 Sub 1 (FL3 Pk Out) (J6)	Mux and Scope (J18)
Dual FL (FL2 Out) (J7)	Mux and Scope (J19)
Dual FL (2Log Out) (J8)	Mux and Scope (J20)
Dual FL (FL2 Pk Out) (J6)	Mux and Scope (J21)

***Note:** Text contained in parentheses indicates silkscreen information on circuit cards, NOT cable labeling.*

Software Installation

Verify that system software is at 4.01 or above. If not, obtain and install current software. See Heading 4.9, SOFTWARE INSTALLATION for instructions.

System Test

1. Remove the 488 DL filter and replace with a full mirror.
2. Remove the following filters for this test so that only 45° filter elements are present in PMT block: 488 LB, 525 BP, 575 BP, 675 BP, 488 BP.
3. Reset those cards where coax cables were removed and installed from.
4. Turn instrument power ON.
5. Ensure column for 5th PMT HV INT Gain PK Gain is present and operational.
6. At the Control Align screen, select:
   a. **Align LED**.
   b. **Pulse Mode**.
   c. **100% Drive**.
   d. **1 kHZ**.
7. At the Scope Screen:
   a. Select **PMT 2 Peak** for Trace 1.
   b. Select **PMT 5** Peak for Trace 2.
   c. Change PMT 2, 3, 4, 5 Int Gain to 30.
   d. Change PMT 2, 3, 4, 5 Peak Gain to 30.
   e. Increase PMT 2 HV for a 3 Division Tall Peak Pulse on Trace 1.
   f. Increase PMT 5 HV for a 3 Division Tall Peak Pulse on Trace 2.
8. At the Scope Screen:
   a. Select **PMT 2** Peak for Trace 1.
   b. Select **PMT 5** Peak for Trace 2.
   c. Change PMT 2 and PMT 5 Peak Gains all around and ensure the amplitudes follow as expected. Put Gain back to 30.
9. At the Scope Screen:
   a. Select **PMT 3 Peak** for Trace 1.
   b. Select **PMT 4 Peak** for Trace 2.
   c. Increase **PMT 3 HV** for a 3 Division Tall Peak on Trace 1.
10. Insert the 575 BP filter in the slot under PMT 4.
11. Increase PMT 4 HV for a 3 Division Tall Peak on Trace 2.
12. Change PMT 3 Peak and PMT 4 Peak Gains all around and ensure the amplitudes follows as expected. Put Gains back to 30.
13. At the Scope Screen:
    a. Select **PMT 2** for Trace 1.
    b. Select **PMT 3 Int** for Trace 2.

14. At the FL Compensation Screen:
    a. Select **PMT 2 minus PMT 3 Int** for 50% and ensure Trace 1 decreases by 50%. Put back to 0%.
    b. Select **PMT 2 minus PMT 4** for 50% and ensure Trace 1 decreases by 50%. Put back to 0%.
    c. Select **PMT 3 minus PMT 2** for 50% and ensure Trace 2 decreases by 50%. Put back to 0%.
    d. Select **PMT 3 minus and PMT 4** 50% and ensure Trace 2 decreases by 50%. Put back to 0%.
15. Replace 575 BP with 640 DL filter under PMT 4.
16. At the Scope Screen:
    a. Select **PMT 4 Peak** for Trace 1.
    b. Select **PMT 3 Peak** for Trace 2.
17. Place the 550 DL filter under PMT 3. Check for three division tall pulses for 3, 4, and 5. Adjust HV if necessary.
18. At the FL Compensation Screen:
    a. Select **PMT 4 minus PMT 3** for 50% and ensure Trace 1 decreases by 50%. Put back to 0%.
    b. Select **PMT 3 minus PMT 4** for 50% and ensure Trace 2 decreases by 50%. Put back to 0%.
    c. Put **PMT 5 Peak** on Trace 2.
    d. Select **PMT 5 minus PMT 4** for 50% and ensure Trace 2 decreases 50%. Put back to 0%.
19. Put all light filters back in proper positions in slot.
20. Turn off **Align LED**.

3.12 BEAM TRANSLATOR UPGRADE

Beam translators are available as upgrade parts; the translators **are not** part of the modification kit for the HeCd laser. The customer can order the kit using PN 6912985-4. The kit contains:

- Three Beam Translators, one for each laser
- UV light (either HeCd or Argon UV laser, PN 6858244-0
- 488nm light (Argon laser), PN 6858245-8
- 633nm light (HeNe laser), PN 6858243-1
- Three micro optical rails for mounting, PN 3814206-2
- Two machine screws for each rail that mounts to the optical bench, PN 2851763-7

The translator can be mounted as in the attached diagram for use when adjusting the beam to the proper height. The resultant beam is parallel to the exit beam of the appropriate laser. This allows the user to use either co-lateral or separate beams, depending on the application.

Tools/Supplies Needed

- ❑ Beam Translator Kit, PN 6912985-4
- ❑ Laser alignment targets with three target holes, large, PN 6857274-6, small, PN 6857236-3
- ❑ Mounting screws, PN 2851920-6
- ❑ Fluorospheres

Laser Mounting Procedure

The long dimension of the optical bench is referred to as the columns. The shorter dimension of the optical bench is referred to as the rows.

1. Mount the UV laser in the farthest rear position possible on the table top.

   Note: Allow enough room to adjust the laser XY-axis column 16, row 9 parallel with the long dimension of the optical bench. Allow enough room in front of the laser for optics, including the beam expander/reducer, a target, and the optical rail.

2. Mount the Argon laser in the column 8, 9, and 10, row 7.

   Note: Allow enough room in front of the laser output for the beam expander, a target, and the optical rail.

3. Mount the HeNe laser next to the fluorescence collection assembly.

   Note: Allow enough room for the alignment of the XY-axis of the HeNe laser and the fluorescent collection assembly. Allow enough room in front of the laser output for the beam expander, a target, and the optical rail.

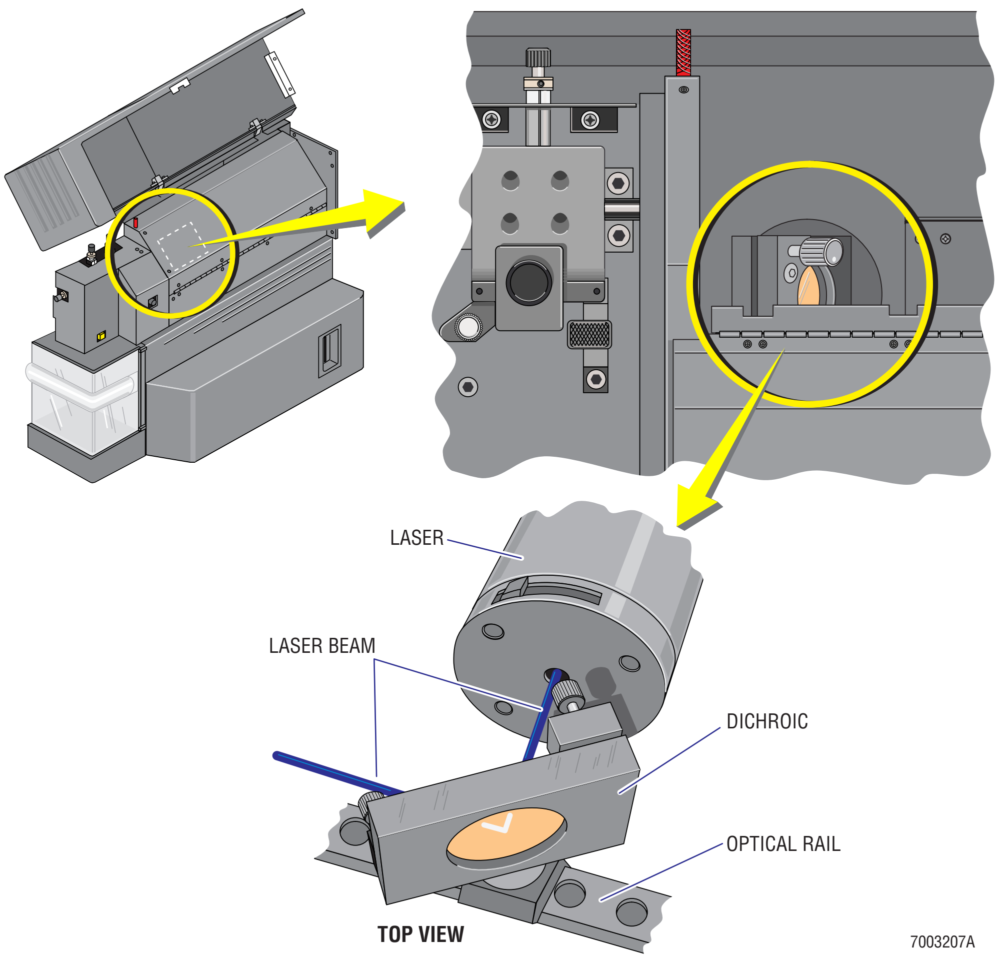
Standard Laser Alignment Procedure

This alignment procedure assumes alignment of the Argon laser on top, and the HeNe and UV lasers on the bottom.

ATTENTION: The order in which you align the lasers may need to be changed. If a dichroic blocks the light from a laser further out on the optical bench, do the dichroic alignment for the interfering dichroic last. For example, if a 488 nm laser in position three is blocked by the 488 dichroic in position two, align the third dichroic before you align the second dichroic.

Figure 3.12-1 shows the laser beam hitting the dichroic.

Figure 3.12-1 Laser Beam Hitting Dichroic, Top View



1. Determine which laser beam's particles will pass through first, and use that laser as a trigger source for the Gated Amp.
2. Determine which lasers are to be used and in what time/space dimension, top beam, or bottom beam.

3. Install the two 5 in. targets on the optical bench in front of the HeNe laser separated by two mounting holes:
    a. Remove the dichroics.
    b. Install the 5 in. targets in front of the first laser (HeNe, HeCd, or other).
    c. Align the laser beam through the 5 in. hole.
    d. Repeat steps a through c for additional lasers.

    Note: Allow enough room to see the alignment holes in the targets.
4. Remove the scatter sensor.
5. Remove the laser shutter.
6. Remove the beam shaper.
7. Raise the flow cell all the way using the flow cell vertical adjustment knob.
8. Install the two smaller laser alignment targets on the vertical optical plate:
    a. Install the small targets.
    b. Install the first dichroic.
    c. Align the first laser through the small target holes.
    d. Repeat steps a through c for additional lasers.
9. At the Cytometer's Control/Align screen, turn on the HeNe and air-cooled Argon lasers.
10. Turn on the UV laser using the switch located on the laser.
11. Close the output shutters on the air-cooled Argon and the UV lasers to prevent beam over-exposure.

    Note: At this point, only the HeNe laser should be emitting a beam.
12. Align the HeNe laser:
    a. Use the 90-degree dichroic mirror to align the HeNe through the two vertical optical plate-mounted-targets.
    b. Close the shutter on the HeNe laser.
13. Move the two optical bench mounted targets to the front of the air-cooled Argon laser.
14. Open the shutter on the air-cooled Argon laser.
15. Align the Argon laser:
    a. Use the 90-degree dichroic mirror to align the Argon through the two vertical optical plate-mounted-targets.
    b. Close the shutter on the air-cooled Argon laser.

WARNING Risk of personal injury due to ultra-violet laser beam exposure. Remove rings and other metal jewelry before continuing. The laser beam can cause eye damage if viewed either directly or indirectly from reflective surfaces (such as a mirror or shiny metal surface, including tools). Avoid direct exposure to the beam. Do not view directly or with optical instruments, except for special service instruments as directed in the service manual. Wear latex surgical gloves to reduce the amount of UV in the event you accidentally place your hands in front of the beam.

16. Open the laser output shutter on the UV laser.

17. Align the UV laser:
    a. Use the 90-degree full mirror to align the UV laser through the two smaller vertical optical plate-mounted-targets.
    b. Use a business card or fluorescent “day glow” paper to detect the UV laser position.
18. Close the laser output shutter on the UV laser.
19. Place the screw-on targets (input and output lenses) on the beam reducer.

    Note: Older style beam reducers can be used, but there are no built-in targets.
20. Install the beam reducer on the optical bench as close as possible to the output of the UV laser.

    Note: Allow enough room to remove the screw-on targets from the beam reducer when you are finished.
21. Open the UV laser output shutter.
22. Align the X-, Y-, Z-axis of the beam reducer through:
    a. The two optical bench mounted targets.
    b. The two vertical optical plate mounted targets.
23. After the beam reducer is properly aligned, remove the screw-on targets from the beam reducer.

Collinear Laser Alignment, Fine Tuning Procedure

1. Remove the two 5 in. (13 cm) optical bench mounted laser alignment targets and the two vertical optical plate mounted targets.
2. Install the scatter sensor.
3. Install the beam shaper.
4. Install the laser shutter.
5. Use the flow cell vertical adjustment knob to lower the flow cell to its proper position.
6. Verify the proper position of the flow cell by using the camera to view placement.

   Note: Remove the 525 BP filter to obtain a side scatter/fluorescent signal in PMT2.
7. Except for the 1.0 ND filter, remove all optical filters from the forward scatter sensor filter holder.
8. Install the filter holder back in the FALS Sensor.
9. Press **SHEATH** to initiate sheath flow.
10. Obtain traces on Cytometer’s Scope screen.
    a. Choose **Fals Peak** for lower trace.
    b. Choose **PMT 2** Peak for upper trace.
11. Open HeNe laser output shutter.
12. Run DNA-Check Beads at a data rate of approximately 100.
13. Align optical area for maximum signal on both traces of Digiscope:
    a. Flow cell X/Y Adj. and Rotation
    b. Beam shaper X/Y/Z Adj.
    c. Fluorescence collection assembly focus (snout).

14. Open the Argon laser output shutter.

ATTENTION: Do not change any flow cell or beam shaper alignment, otherwise you will undo the alignment that was just completed for the HeNe.

15. Use the Argon laser 90 degree dichroic adjustments to align for maximum co-linear signals, where both Argon and HeNe signals appear on top of each other on both traces of the Digiscope.
16. Close the Argon laser output shutter.
17. Open the UV laser output shutter.

ATTENTION: Do not change any flow cell or beam shaper alignment, otherwise you will undo the alignment that was just completed in step 13.

18. Align for maximum co-linear signals on both traces of the Digiscope using the 90 degree mirror only.
19. Open the Argon laser output shutter.
20. Block each laser off, one at a time, with a target or opaque object. Observe the change in displayed signals on the Digiscope.

Note: Now that all three lasers are active, be sure all three resultant signals are occurring in the same time frame. This means the signals should be co-linear, where all three appear to be on top of each other.

Time/Space Separated Laser Alignment Procedure

1. Close the Argon laser output shutter.
2. Set the UV laser power up/or down to create a distinguishable difference in amplitude between the HeNe and UV Laser - resultant signals.
3. Install the adjustable beam translator between the HeNe laser and its adjustable dichroic filter.

   Note: Allow enough space between the translator and the optical rail to mount a single laser target.
4. Verify that the:
   a. Translator knobs face away from laser.
   b. Translator window is perpendicular to the optical bench.
5. Adjust translator height in post for laser to penetrate as close to the center of the translator window as possible.
6. Refer to the multi-hole target and set the Alignment mode for top(1). Move the pin on bottom of target to line up with the desired target hole, and lock down with a setscrew.
7. Install the middle target in the space between the beam translator and the HeNe dichroic, and ensure the target is square with the table top.
8. Rotate the translator window until the HeNe passes through the middle of the target hole.
9. Remove the HeNe laser target.
10. Use the adjustable HeNe laser dichroic on the optical rail, look at the lower trace on the Digiscope Screen, and adjust the trace between the peak of the HeNe laser-caused signal

and the peak of the UV laser caused signal. The adjustment should be:

- 20 microseconds for a 100 µm quartz tip
- 30 microseconds for a 76 µm quartz tip
- 3.5 microseconds for a 76 µm jet-in-air tip.

Note: The UV signal is on the far left of the Digiscope. Test this by increasing and decreasing the UV laser power and observe the far left peak that follows.

11. Close the HeNe laser output shutter.
12. Install the adjustable beam translator between the Argon laser and its adjustable dichroic.

    Note: Allow enough room between the translator and the optical rail to mount a single laser target.
13. Verify that the:
    a. Translator knobs faces away from the laser.
    b. Translator window is perpendicular to the optical bench.
14. Adjust the translator height in post for the laser to penetrate as close as possible to the center of the translator window.
15. Refer to the multi-hole target and set the alignment mode for "lowest" target hole.
16. Move the pin on the bottom of the target to line up with the desired target hole, and lock down the pin with setscrew.
17. Install the lowest target in the space between the beam translator and the Argon dichroic, and ensure that the target is square with the table top.
18. Rotate the translator window so that the Argon beam is aligned with the lowest laser target hole.
19. Remove the lowest Argon laser target.
20. Use the adjustable Argon dichroic on the optical rail, look at the lower trace on the Digiscope Screen, and adjust the trace between the peak of the Argon-caused signal and the peak of the UV-caused signal. The adjustment should be:
    - 20 microseconds for a 100 µm quartz tip
    - 30 microseconds for a 76 µm quartz tip
    - 3.5 microseconds for a 76 µm jet-in-air tip.

    Note: The UV signal is the middle one on the Digiscope. Test this by increasing and decreasing the UV laser power and observing the far middle peak that follows. At this point, the beams should be Argon, UV, and HeNe.
21. Refer to the multi-hole target and set the Alignment mode for "highest" hole.
22. Move the pin on the bottom of target to line up with desired target hole, and lock down the pin with a setscrew.
23. Install the highest target in the space between the beam translator and UV mirror on the optical rail, and ensure the target is square to the table top.
24. Rotate the translator window so the UV beam is aligned with the top hole in the alignment target.
25. Remove the raised UV laser target.

26. Use the adjustable UV laser mirror on the optical rail, look at both traces on the Digiscope Screen, and adjust the UV beam using the 90-degree UV mirror for the same time frame as the HeNe-caused signal. Between the peak of the UV and HeNe and the Argon-caused signals the time separation should be:
    - 40 microseconds for the 100 µm sort sense tip at 12 psi
    - 60 microseconds for the 76 µm sort sense tip at 12psi, or
    - 7 microseconds for the 76 µm jet-in-air tip.

    Note: The Argon laser signal should be on the far left of the Digiscope screen. Test this by increasing and decreasing the UV laser power and observing the far right peak that follows. Block the HeNe laser and raise/lower the Argon laser power and observe the far left signal that follows.
27. Determine correct alignment by ensuring the Argon laser exits near the center of the beam shaper and the UV laser exits the beam shaper slightly below the Argon. Place a business card several different places in the beam's path to verify the beams remain parallel through the flow cell all the way to the FALS sensor.

    Note: Lower laser power is easiest on the eyes. It is recommended to place it right after the beam shaper, and just before the scatter sensor.

3.13 SORT PERFORMANCE (ESP) UPGRADE

Purpose

Do this procedure to bring existing instruments to the correct revision level and to install the sort enhancement kit.

Do this procedure only on systems when installing the Sort Upgrade (ESP) kit.

Tools/Supplies Needed

- ❑ Sort Upgrade Kit
  - PN 6913253 for Elite Systems without switch-amps (before SN U07122) and for Elite Systems with the Gated Amp option. This kit includes switch-amps.
  - PN 6913250 for Elite Systems that have Switch-Amps (after SN 707122) and for Elite Systems with the Gated Amp option. This kit does not include switch-amps.
- ❑ Sort Delay R3 card, PN 6706034
- ❑ Sort Oscillator R2 card, PN 6705694
- ❑ Pulse Pileup Det./TOF card, 6705954
- ❑ Sort bracket, PN 6856321
- ❑ Control panel, PN 6858680

Preliminary Procedure

1. Verify that the kit is correct and complete.
2. Verify that:
   a. The software is version 4.0 or higher.
   b. Watchdog Timing has been done.
   c. Pneumatic Reliability Improvement, Heading 4.5, PNEUMATICS SYSTEM, has been done for low-bleed regulators.
   d. The instrument is equipped with a 100 µ tip and 3000 V power supply.
3. One at a time, remove each card listed below from the system and examine it for revision level as listed in Table 3.13-1.

   ATTENTION: If the revision level is not correct, then return the card to the authorized repair center. Do not make changes in the field.

Table 3.13-1 Circuit Card Revision Level

Circuit Card	Part Number	Minimum Revision Level	Task
Peak Scatter/Mux	6705369	C	Ensure C98 is 3 pF Ensure C99 is 10 pF Ensure R67 is 100 ohms
3 PMT Sub Amp	6705246	B	Ensure capacitor locations C136, C134, C132 are not present. Ensure C114, C85, C58 are 5 pF. Ensure C133, C135, C137 are 24 pF.
Scatter Sensor	6705182	B	Ensure U1 is a OP61A.
PMT Gated Amp	6704008	D	Ensure capacitor locations C147, C193,C59, C24 are not present. Ensure diode locations CR19, CR14,CR11, CR4 are not present. Ensure U18 is AD746. Ensure R205 is 1000 ohm. Ensure R40 is 4.99 kohm. Ensure R36 is 10 kohm. Ensure that near U41, U30, U19, U10, a diode was added to the card near each of these op amps. The diode's anode is electrically connected to pin 8. All diode cathodes are connected to ground.
Scat/Aux Gated Amp	6704009	D	Ensure capacitor locations C98, C67,C41,C20 are not present. Ensure diode locations CR19, CR10,CR15, CR6 are not present. Ensure U1 is AD746. Ensure R143 is 1000 ohm. Ensure R1 is 4.99 kohm. Ensure C109 is 5 pF. Ensure that near U23, U16, U11, U7, a diode was added to the card near each of these op amps.
Gated Amp Control	6705327	C	Ensure diode location CR5 is not present.

See Table 3.13-2 for additional information.

Table 3.13-2 Additional Information on Cards

Card Name	Description	Minimum Revision Level
Peak ADC	Replaces ADC card PN 6704139	B
Peak Scatter Sensor	Located on scatter sensor	B
Gated Amp Control	In all units	C
Scat/Aux Gated Amp	Gated Amp units only	D
PMT Gated Amp	Gated Amp units only	D
Quad PSH	2 used	F
3 PMT Sub Amp	1-3 used	B
Peak Scatter/Mux	In all units	C

Sort Kit Installation

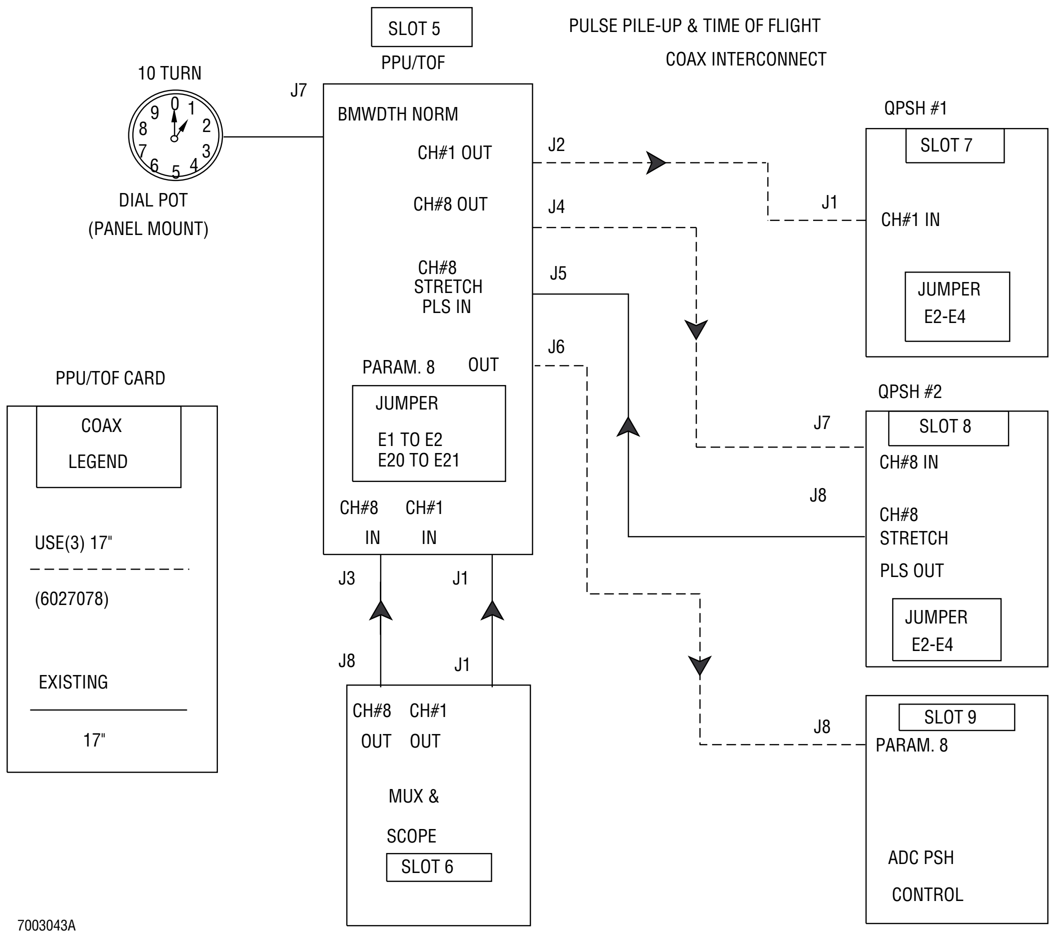
1. Do Heading 5.1, VERIFICATION INSPECTION PROCEDURE to ensure instrument is 100% operational.
    a. Record all HV and Gain settings.
    b. Perform actual sorts with beads, Cyto-Trol Control cells, or cells to determine baseline sorting capability of the unit before beginning upgrade.
    c. Make printouts of pre- and post-sort histograms indicating purity and sort/acquisition rates.
2. Remove the Sort Delay card and replace it with the Sort Delay R3 card.
3. Remove the Sort Oscillator card and replace it with the Sort Oscillator R2 card.
4. Install the:
    a. Pulse Pileup Det./TOF card
    b. Sort bracket
    c. Control panel.
5. Turn the instrument off, and remove the right front panel to expose the card racks.
    a. Insert Pulse Pileup Det./TOF card in bottom (Data Acquisition) card cage slot 5.
    b. Install coax cables as shown in Figure 3.13-1.
    c. Replace existing cards with cards in upgrade kit.
    d. Remove the two upper right screws securing the lower card rack, and remove the lower right screw securing the upper card rack.
6. Use the screws removed in step d to secure the new control panel.
7. Connect the multipin connector to the Sort Oscillator card R2.
8. Connect the cable terminating with a bullet connector to the TOF/PPU card J7.
9. Temporarily hold the new front panel up to the instrument and verify that the new cutout in the panel provides access to the controls when the panel is in place.
10. Verify instrument operation:
    a. Do TOF Verification under Heading 5.1.
    b. Do Heading 4.2, SORT WAVEFORM VERIFICATION AND ADJUSTMENT PROCEDURE.
    c. Do Sort Coincidence Verification under Heading 4.2.
11. Install the front panel.
12. Install the Piezoelectric Transducer. See Heading 3.16, PIEZOELECTRIC TRANSDUCER INSTALLATION for the Piezo assembly included in the sort kit.

    Note: If the Piezo is already installed, return the part from the Sort kit.
13. Do Operational Test - Cytometer Electronics, Optics, or Fluidics under Heading 5.1.

    Note: FALS gain setting must be set higher to achieve the same mean channel obtained before the update. You will also note differences in the fluorescence means. This is normal.
14. Do Sort Function Verification under Heading 4.2.

15. Sort with beads, Cyto-Trol Control cells, or cells. Make a set of printouts showing pre- and post- sort histograms indicating sort purity and sort/acquisition rate(s) to give to the customer.

Figure 3.13-1 Coax Cable Connections



ESP-Equipped Units

Table 3.13-3 summarizes the upgraded circuit cards on ESP units.

Table 3.13-3 Circuit Cards in ESP-Equipped Units

Card Name	Description	Minimum Revision Level
Pulse Pileup Det./TOF	New card	N/A
Sort Delay R3	New card	N/A
Sort Oscillator R2	New card	N/A
Peak ADC	Existing card	B
Peak Scatter Sensor **Note:** This is on the Scatter Sensor.	Existing card	B
Gated Amp Cont	Existing card	B
Scat/Aux Gated Amp	Existing card	D
PMT Gated Amp	Existing card	D
Quad PSH	Existing card	F

Note: For any pot or cable problem, order the sort sub-assembly. The panel sub-assembly is the front panel with the cutout for the new control panel.

3.14 ENTERPRISE LASER UPGRADE

Purpose

This procedure provides information for attaching and adjusting the optics assembly used with the Coherent Enterprise laser. Installation and adjustment information for the laser is contained in the laser manual, PN 7232520-6.

The laser light exiting the Enterprise laser contains both the 488 nm and the UV (351 nm) wavelengths. The Enterprise laser is operated in light regulation relative to the UV. There is no separate adjustment for the visible output.

The Elite optical system must separate the two colors, provide attenuation for the visible beam, provide a means to focus the UV beam and provide beam translation capability.

The beams from the laser strike the beam splitter which passes the 488nm wavelength and reflects the UV to the UV reflector which directs the beam through the beam expander/reducer. The expander/reducer can be adjusted to make the UV beam spot size compatible with the 488 nm beam spot size.

The 488 nm beam passes through the attenuator wheel. The wheel is a variable density filter that can be set to give from 0 to ND2 attenuation (divide the 488 beam power by a factor between (0-100). Both beams pass through the translators. These translators can be easily adjusted to independently shift the height of the two laser beams without affecting their angular alignment.

Tools/Supplies Needed

- ❑ Enterprise laser kit
- ❑ Enterprise laser customer manual, PN 7232520-6

Note: Installation of this laser is identical to installation of the I305 laser except for the laser.

WARNING Risk of personal injury. You must be certified as trained on the Coherent I90, I60 or I300 lasers and you must be in contact with a technical support person who is trained on the Enterprise laser in order for you to qualify as an authorized installer.

If, and only if, you meet these requirements, read the manual supplied with the laser, especially Chapter 3, Utility and Environmental Requirements and System Installation, and Chapter 6, Rear Mirror Adjustment.

Call your technical support person before continuing.

WARNING Risk of personal injury. The Enterprise laser always emits UV as well as visible radiation. Take appropriate precautions to avoid exposure. Keep the laser shutter closed and/or block laser beams except when needed. Perform all alignments at minimum power. Align the UV by wearing protective eyewear and using a business card to observe fluorescence from the laser. Avoid skin exposure to any laser beam. Always consider where the laser beams may go before opening the shutter.

Important Operating Notes

- Optimum laser lifetime is achieved with the laser operated at 50 mW UV (in UV regulation).
- When the heat exchanger is used, be sure the hot air exhaust from the exchanger is isolated from the laser head and the Elite system.
- Ensure lab HVAC system can maintain a stable ambient temperature within specified range. Refer to the Coherent Enterprise laser manual(s).
- For best power regulation, lowest noise, and best laser life, the manufacturer recommends operation at 50 mW UV power continually. The ND attenuator can be used to reduce the 488 power levels if desired.
- If the laser is operated at reduced power levels, it is important that it be operated at the high power level for one hour at least once a week. You should discuss the different laser operating modes with the customer to determine how to best meet their needs.
- The laser can be operated in either UV or 488 light regulation. For single beam applications use the appropriate mode. Note that 488 mode is a secondary control loop; the 488 sensor output is compared to the requested 488 power and the error is used to modify the requested UV power. The laser cannot be operated at 488 power levels which are so low as to provide no UV output.
- The laser should NEVER be operated in either regulation mode such that its UV output is less than 10mW.
- For dual beam use, UV regulation appears to be the best choice for optimum UV power stability. Allow 1.5 hours for warmup to stabilize the 488 power.

Installation

1. If the Elite system is not already equipped with the Interlock Relay card and large laser compatible covers, install the interlock system, optical rail and relocate the air cooled lasers.

   Note: If using the Enterprise laser, the air-cooled Argon will not be used; therefore, remove its dichroic.
2. Verify that the output aperture of the Enterprise laser is at hole 12, 3. See Figure 3.14-1.
3. Warm the laser up at 50 mW UV for 30 minutes.
4. Install two 5 in. targets on the table so their apertures are above row three.
5. Adjust the laser so the beam passes through the targets. The goal is to have the beam parallel with and 5 in. (cm) above the row.
6. Clamp the laser feet to the table using the supplied hardware.
7. Remove the targets and install the beam separator with two bolts at the position shown in Figure 3.14-2.
8. Install the 5 in. targets at 1,3 and 1,6 so the apertures are at these coordinates.
9. Set the two beam translators to be vertical and straight to introduce no beam translation.
10. Adjust the positioner knobs to target the UV beam.

    Note: Further adjustments depend on the customer's specific operating needs: the beam translators can be used to shift the beams to be coaxial or to place either beam in the upper position. As an initial test, align the beams to be coaxial.

Figure 3.14-1 Enterprise Laser Mounting Location

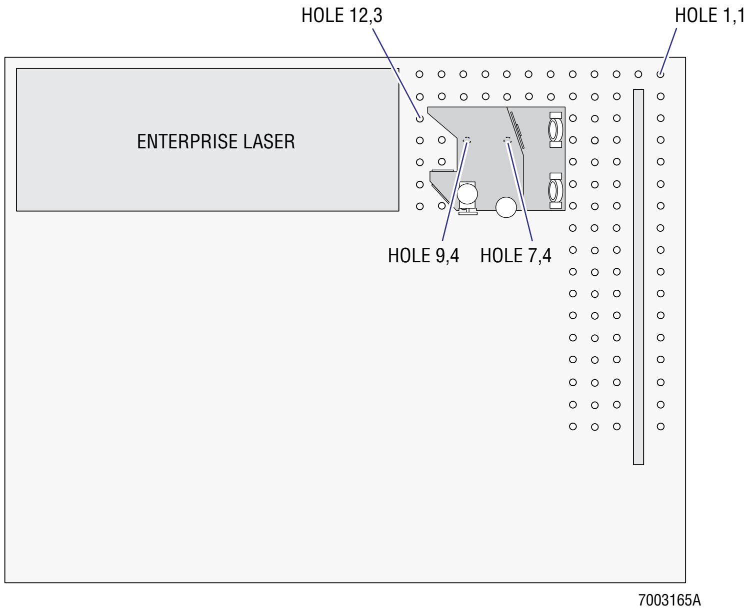
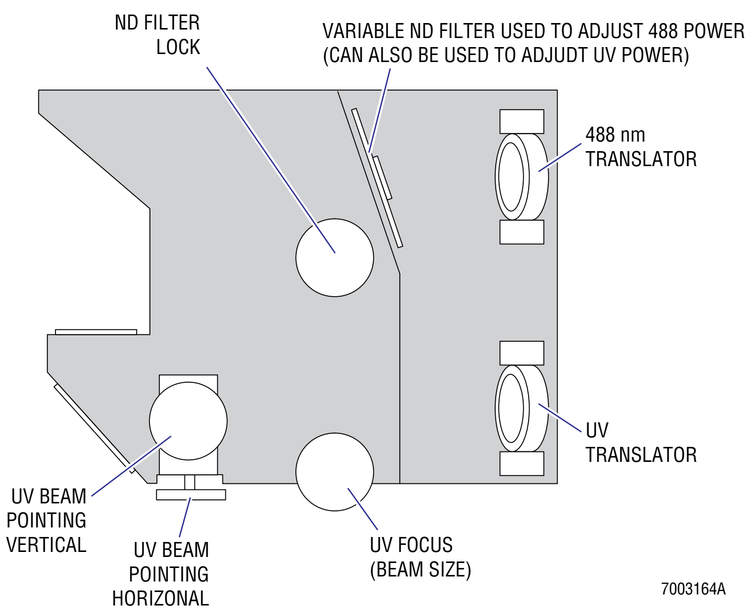


Figure 3.14-2 Beam Separator Location



11. Install the short targets in the flow cell area.
12. Remove the tall targets and install the optical rail elements.
13. Remove the flow cell tip and beam shaping optics.
14. Begin with the front (HeNe) laser, use the optical rail beam positioners to target each beam. Be sure all adjustments are secure when done.
15. Reinstall the flow cell, beam shaping optics and scatter sensor.

16. Remove all targets.
17. Block all beams except the 488, and align the flow cell and beam shaper using fluorescent particles. Adjust the beam focus for narrowest peak fluorescence.
18. Block the 488 beam and allow the UV beam to enter the flow cell.

    Note: You can monitor the beam's location by holding a business card between the flow cell and scatter sensor.
19. Make slight adjustments to the optical rail UV beam mirror until you see a scatter or GFl signal. Adjust for optimum amplitude.
20. Compare the UV beam and 488 beam peak pulse width. Adjust the UV beam width on the beam separator to match the 488 pulse width. This disturbs the UV beam alignment; compensate as needed.
21. Block the 488 and UV beams and adjust the HeNe laser mirror for peak scatter.
22. Connect the laser interlock cable between the laser power supply and the heat exchanger interlock cable.

    Note: If the system operates with tap water, connect the laser interlock cable between the laser power supply and the heat exchanger interlock defeat.
23. Verify that the Enterprise laser shuts down when the Laser ON/OFF key switch on the Elite system is turned to OFF or when the laser compartment door is opened.
24. Replace all covers.

3.15 COLLECTION OPTICS UPGRADE

Purpose

Use this procedure to upgrade flow cell tips. New flow cell tips and collection lens assemblies are available as a purchased upgrade for existing Elite systems. Systems manufactured after September 1993 incorporate these items as standard. These are readily identified by noting that the new collection lens is noticeably smaller. The new flow cells have a smaller lens and no mirror. The flow cell tip types are:

- Flow cell tip 100 µ 3x
- Flow cell tip 76 µ 3x
- Flow cell tip Biosense

The new flow cell tips can be used without replacement of the collection optics assembly. This does require removal of the collection lens focus stop to allow sufficient focusing adjustment.

Tools/Supplies Needed

- ❑ One of the following flow cell tips:
  - ▸ Flow cell tip 100 µ 3x, PN 6859300-0
  - ▸ Flow cell tip 76 µ 3x, PN 6859313-1
  - ▸ Flow cell tip 3x, PN 6859341-7
- ❑ Pinhole snout 3x, 6859315-8
- ❑ Snout Upgrade kit, PN 6913251-1
- ❑ Power meter

Flow Cell Replacement Procedure

Note: If system does not have the 3x collection lens assembly remove the collection lens focus stop on the side of the PMT housing. Caution the customer not to strike flow cell with collection lens.

1. Remove the existing flow cell tip.
2. Install the new flow cell tip.

Snout Upgrade Procedure

Purpose

Removal of the fluorescent pickup lens assembly first requires removal of the vertical optical plate. This is the plate on which the FALS detector, the beam shaping stage and the flow cell adjustment stage are mounted. Although the front of pickup lens protrudes through this plate, the lens assembly mounting flange will not pass through the plate opening. The following procedure must be followed to ensure correct reinstallation of the optical plate.

Tools/Supplies Needed

- ❑ Allen wrench, 5/32 in. (long or T-handle recommended)
- ❑ Allen wrench, 7/64 in. (long or T-handle recommended)

Removal Procedure

If materials are available, it is highly recommended that a sensitivity test be performed before and after collection lens replacement. As a minimum, collect CV data with fluorospheres. Note the HV and gain. If cells or Cyto-Trol Control cells are available, run samples stained with red and green dyes before and after collection lens replacement.

1. Do a sensitivity test:
   a. Collect CV data.
      - If cells or Cyto-Trol control cells are available, run the sample stained with the red and green dyes.
      - If cells or Cyto-Trol control cells are not available, run fluorospheres.

   b. Record the HV and gain.
   c. Continue the upgrade procedure:
      - If doing this procedure as instructed in step 10 of the Replacement Procedure, go to step 11 of that procedure.
      - If doing this procedure for the first time, go to step 2.
2. Allow the Argon laser to warm up for at least 30 minutes.
3. Remove the FALS detector and install alignment target at this location.
4. Remove pneumatically operated beam shutter and install second alignment target at this location.
5. Remove the flow cell tip, beam shaping lens, sample tubing and sample drawer.

ATTENTION: This alignment must be done very carefully. It may be helpful to use a power meter to measure and record the laser power passed through the targets. This will give you a reference value to which you should return to when you replace the plate.

6. Adjust the mounting hardware of the Argon laser as needed to perfectly center the laser beam through the two target apertures. Be sure laser is securely mounted when done.
7. Block the laser beam with a tall target or other nonreflecting metallic object.

   Note: Be sure not to disturb laser alignment. Do not turn laser off.
8. Ensure that crystal drive is set to 0.0%, and disconnect electrical connector to the Bimorph (crystal drive)
9. Ensure that deflection is set to 0.0%, and:
   a. Disconnect the alligator clip from the flow cell sample introduction tube.
   b. Disconnect the ground connector from the same cable at the deflection plate holder.
10. Using a scribe or permanent marker, mark the table top at the edge of the plate to provide a reference for horizontal plate location with reference to the table top.
11. Locate the six Allen-screw bolts that secure the vertical optical plate to the PMT housing assembly. There are four bolts forming a vertical line between the flow cell and lens stages. There are two bolts at the lower edge of the optical plate.
12. Remove the six bolts located in step 11.
    a. Note the location of the shorter bolt. Do not allow optical plate to fall.
    b. Carefully set aside optical plate.

13. Remove the pickup lens assembly by removing the four bolts securing the pickup lens assembly to the PMT housing.

Replacement Procedure

1. Attach the replacement pickup lens to the PMT housing with the four bolts removed in step 13 of the Removal Procedure.
2. Reinstall the optical plate using the 6 bolts removed in step 12 of the Removal Procedure.
3. Align plate with mark made in step 10 of the Removal Procedure.
4. Tighten bolts slightly.
5. Remove laser block you installed in step 7 of the Removal Procedure, allowing the beam to strike the target(s) on the optical plate.
6. Verify the laser beam passes cleanly through the target apertures:
    a. Loosen the plate mounting bolts enough to shift the plate position until the laser beam passes cleanly through the target apertures.
    b. Tighten the bolts securely and check the that the beam passes through the targets again.
    c. Repeat step a and b until proper alignment is achieved again.

    Note: If you used a power meter as suggested before step 6 of the Removal Procedure, ensure that the laser power past the apertures is the same or better than before.
7. Reinstall the:
    a. FALS detector.
    b. Beam shutter.
    c. Flow cell tip.
    d. Beam shaping lens.
    e. Sample tubing.
    f. Sample drawer.
8. Reconnect:
    a. The electrical connector to the bimorph (crystal drive).
    b. The ground connector at the deflection plate holder.
9. Do Heading 5.1, VERIFICATION INSPECTION PROCEDURE.
10. Repeat step 1 to do the Sensitivity test.
11. Ensure the customer is satisfied with the upgrade.

3.16 PIEZOELECTRIC TRANSDUCER INSTALLATION

Purpose

Do this procedure to install the Piezoelectric (Piezo) transducer in instruments manufactured **before March 1993**.

Tools/Supplies Needed

❑ Stage plate flow cell kit, PN 6912795-9, which includes:
- Stage plate flow cell, PN 6858242-3
- Flow cell with intro rod, PN 6858366-7
- Cap (nut), PN 1021202-2
- Tubing, silicone 0.015 in (0.04 cm) PN 3213154-9

Installation

1. Turn off the instrument.
2. Remove the flow cell tip and set it aside.
3. From the flow cell, remove the:
   a. Sheath line.
   b. Vacuum line.
   c. Sample line.
4. Unplug the bimorph (crystal drive) connector.
5. Remove the two screws that secure the flow cell mount to the flow cell stage.
6. Use the screws you removed in step 5 to now attach the Piezo assembly to the same threaded holes from which the old assembly was removed.
7. Install the new flow body.
   a. Attach the new flow body to the Piezo assembly.
   b. Attach the flow cell tip from step 2 to the new flow body.
   c. Connect the sheath, vacuum, and sample tubing to the new flow body.

   Note: The kit includes a length of 0.015 in. tubing you can use to replace the existing 0.010 in. tubing.
8. Plug the bimorph connector into the prongs of the Piezo labeled “+” and “-”.
9. Verify that the cable protrudes from the instrument.

   Note: This is the opposite orientation from how the cable was attached to the old bimorph assembly.
10. Do Initial Flow Cell Alignment below.
11. Install the beam shaper and do an optical alignment as instructed in the Special Procedures and Troubleshooting manual.
12. Do Heading 5.1, VERIFICATION INSPECTION PROCEDURE.

Initial Flow Cell Alignment

1. Remove the beam shaper.
2. Verify that the laser beam is centered on the scatter sensor. If not, or if there is any doubt as to laser alignment, install the targets and align the laser.
3. Turn Sheath on and open the laser shutter.
4. Adjust the flow cell vertically to ensure the laser beam is passing through the quartz cell.
5. Rotate the flow cell so the laser beam reflects back through the laser:
    a. Loosen the nut that secures the flow cell to the bimorph.
    b. Rotate the flow cell as needed.
    c. Tighten the nut.
6. Adjust the flow cell tilt so the laser beam reflects back through the laser shutter:
    a. Loosen the two bolts that secure the flow cell stage to the vertical plate only enough to allow the flow cell to be tilted.
    b. Adjust the flow cell tilt as needed.
    c. Tighten the bolts.
7. Center the flow channel in the laser beam using the adjustment on the Piezo assembly.
8. Observe the reflected laser beam and path of the beam through the flow cell.
9. Repeat the above adjustments to ensure:
    a. The laser beam reflected from the flow cell is coincident with the incoming laser beam.
    b. The laser beam is centered on the flow channel.

3.17 WATCHDOG TIMING

Purpose

This procedure extends the watchdog time-out and prevents momentary dropouts in 24 V power, which can occur when the Cytometer CPU is busy.

Do this procedure when Elite Software Version 4.0 is installed.

Tools/Supplies Needed

- ❑ 221 kOhm resistor, PN 4717745-6
- ❑ 22 uF capacitor, PN 1515017-3

Procedure

1. Remove the Sensor Interface card from the instrument.
2. Locate R77 and C44, located about 4 in. (10 cm) back from J9, between U21 and U22.
3. Replace C44 with the 22 µf capacitor.
4. Remove the jumper from E25 - E26.
5. Reinstall the Sensor Interface card.
6. Do Heading 5.1, VERIFICATION INSPECTION PROCEDURE to ensure the instrument's fluidics are normal.

3.18 SOFTWARE VERSION 4.0 INSTALLATION

ATTENTION: Users of pre-486 computers will experience a reduction in system operating speed.

ATTENTION: Country service organizations must track the installation of Software Version 4.0 in instruments with the Autoclone option. The international implementation status must be reported through the field Corrective Action Status by Country form.

Purpose

Use this procedure to install Elite Software Version 4.0 in all instruments currently with version 3.25 software. Version 3.25 was installed when the Autoclone option was installed. The Autoclone/Version 3.25 serial numbers affected are:

- 00N44015	- 00N44016	- 00S09119
- 00S13191	- 00S35515	- 00S35572
- 00S39664	- 00S39665	- 00S44740
- 00S44743	- 00S52904	- 00T07115
- 00T08151	- 00T30519	- 00T33553
- 00T46776	- 00T46777	- 00T46778
- 00U05081	- 00U08166	- 00U08167
- 00U12302	- 00U18397	- 00U23601
- 00U23602	- 00U32613	- 00U36621
- 00U36623	- 00U40632	- 00U49649
- 00U49650		

This procedure also applies to:

- Elite Analyzer Flow Cytometer, 2819
- Elite Software (single), 2365
- Elite Software (multiple), 2366
- High SN ORS09119
- Low SN V39054.

Note: For Elite Analyzers operating with version 3.0 or 3.1 software, version 4.0 is considered an enhancement.

Version 4.0 software is required and should be installed on instruments that include the Panelyzer option. Installation of additional options may require version 4.0 software.

Installation in stand-alone workstations can be done by the customer.

Tools/Supplies Needed

- ❑ Version 4.0 Software Kit, PN 6912816, which includes:
  - Disk 1, PN 6414976-8
  - Disk 2, PN 6414977-6
  - Field Correction Notice, PN 4236307-3

Procedure

1. Insert the **Program Distribution Disk 1 of 2** disk in the 3.5 in. floppy drive.
2. At the DOS command:
   a. Type `B:` and press Enter.
   b. Type `INSTALL` and press Enter.
3. Follow the instructions that appear on the screen.
4. Remove the disk from the floppy drive.
5. Reboot the computer by pressing Ctrl+Alt+Delete.

   Note: if you exit from the Elite program, type `CYTOMETER` and press Enter.
6. Refer to the README.DOC file for additional instructions.
7. Transfer the new control file to the Cytometer by selecting **Acquisition ▸▸ Start** at the Workstation.
8. Do Heading 5.1, VERIFICATION INSPECTION PROCEDURE.
9. Provide the customer with a completed Field Correction Notice.
10. Verify that the customer's manual is updated.

    Note: Updated manuals are also included in PN 6913228-6.

3.19 SOFTWARE VERSION 4.01 INSTALLATION

ATTENTION: This procedure is **mandatory**.

Purpose

Do this procedure to install Elite Software Version 4.01 in all instruments currently with version 4.0. As noted in the README.DOC file, version 4.01 contains several enhancements, including:

- A change in the waste level sensor logic to provide fail-safe monitoring of the level sensing circuits.
- The software does not lock up when Acquisition is started and Sort Logic is changed on the Cytometer Sort screen from Ext Sort to Cmp Abort and Acquisition is stopped (user then goes to Sort screen at the Workstation.)
- The software does not lock up when the user prints a bar code label for a Panelyzer run under the Acquisition application then switches to a different application then back to Acquisition.
- The software reads FCS 2.0 listmode and histogram files.
- The software reads large listmode file (>10 MB). During data analysis, statistics display all asterisks when an overflow in a channel occurs (>65535 events in 1 channel) or when an overflow is a histogram occurs.
- The software allows the user to switch PPU sensitivity ranges independently from sort logic.

This procedure applies to:

- Elite Flow Cytometer, 2362
- Elite Analyzer Flow Cytometer, 2819
- Elite Flow Cytometer with Gated Amp and/or Autoclone Sorting Option, 2360
- Elite ESP Flow Cytometer, 2358
- Elite ESP Flow Cytometer with Gated Amp and/or Autoclone Sorting Option, 2357
- Elite ESP Flow Cytometer with Gated Amp and/or Autoclone Sorting Option and Advanced Lasers, 2356
- High SN W48072
- Low SN V39054

Version 4.01 detects the current version of software installed on the instrument, and if version 4.0 is not detected, you cannot install version 4.01. Version 4.01 disks contain an updated ELITE.EXE and Cytometer files which the INSTALL program copies over the version 4.0 files. Unchanged files are not overwritten.

Newly manufactured instruments, beginning at serial number W48072, are shipped with the software upgrade already installed.

Tools/Supplies Needed

- ❑ Elite Software Version 4.01 Upgrade, PN 6913114-0, US Country use only
- ❑ Elite Software Version 4.01 Upgrade, PN 6913060-7, International Use only

Installation Procedure

ATTENTION: Customers can install version 4.01.

1. Verify that:
   a. Elite Software Version 4.0 is already installed on the instrument. See Heading 3.18, SOFTWARE VERSION 4.0 INSTALLATION for installation instructions.
   b. Watchdog Timing has been completed. See Heading 3.17, WATCHDOG TIMING.
2. Power up the instrument.
3. Press F2 to exit to DOS.
4. Insert the 3.5 in. disk in the 3.5 in. floppy drive.
5. Print the README.DOC file for additional information:
   a. Verify that the printer is connected and ready to print.
   b. If your 3.5 in. floppy drive is drive A:
      1) Type `A:\PRINT README.DOC/P` and press Enter.
      2) Type `A:\INSTALL A` and press Enter.
   c. If your 3.5 in. floppy drive is drive B:
      1) Type `B:\PRINT README.DOC/P` and press Enter.
      2) Type `B:\INSTALL B` and press Enter.
6. Transfer the control file:
   a. When the software screen appears, press F9.
   b. Press Y to transfer the file.

   The Cytometer Control screen disappears during the software transfer.
7. Do Waste Bottle Modification for Software Version 4.01.
8. When the Control screen appears, select **POWER UP VALVES** and wait for the valve action to be completed.
9. If the instrument is equipped with an Autoclone Sorting Option, do Calibration Procedure under Heading 3.10, AUTOCLONE SORTING OPTION INSTALLATION to allow for proper drainage in the Sort Collection area.

   Note: Even if the Autoclone Sorting Option is not being used at this time, you must still do the Calibration Procedure.

Waste Bottle Modification for Software Version 4.01

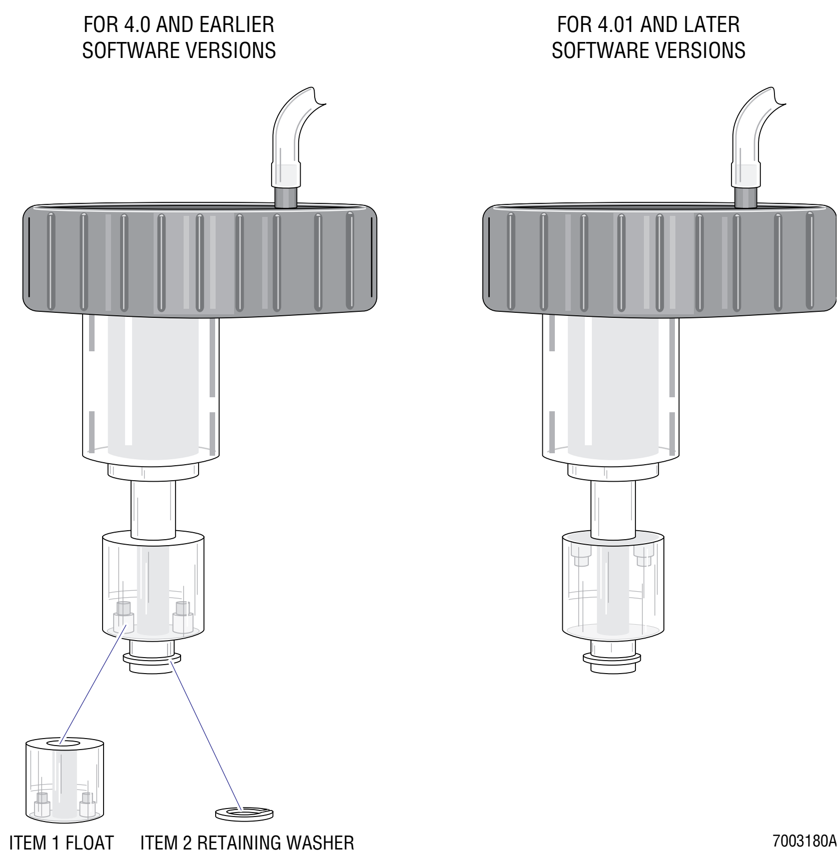
The Elite uses a magnetically actuated switch to detect when the waste bottle is full. In instruments with version 4.0 or earlier software, the switch is closed when the bottle is full. The software interprets this switch closure as “Waste Bottle Full” error condition. If the switch fails or if the user fails to reconnect the switch after emptying the bottle, the bottle full condition may not be detected. Although the instrument incorporates other safeguards, the software logic of version 4.01 detects an open switch condition. This software change, combined with the modification to the level switch, provides a self-monitored system. This means that if the user fails to reconnect the switch or if the switch fails, the *Waste Bottle Full* error message appears on the Cytometer Control screen.

ATTENTION: Software Version 4.01 must be installed before you do this procedure.

WARNING Risk of personal injury. Wear barrier protection including, but not limited to, protective eyewear, gloves, and suitable laboratory attire when performing this procedure.

1. Remove the waste bottle from the instrument:
   a. Disconnect the color-coded connectors.
   b. Disconnect the level detector wires at the bracket.
2. Carefully remove the waste bottle cap from the waste bottle.
3. Look inside the clear plastic float to verify that the two magnets (grayish metallic cylinders) are attached to the bottom of the float. See Figure 3.19-1.

Figure 3.19-1 Waste Bottle Caps



4. Carefully remove the plastic retaining washer. See Figure 3.19-1.
   - The washer has a slit to facilitate removal.
   - Apply sufficient force to remove the washer, being careful not to break it. An extra washer is provided in case of breakage.
   - Do not allow the float to fall to the table or floor.
5. Carefully slide the float off the waste bottle cap.
6. Rotate the float 180 degrees and slide it back onto the waste bottle cap.
7. Verify that the magnets are now attached to the top of the float.

8. Carefully reattach the retaining washer.
9. Reconnect:
   a. The color-coded connectors.
   b. The level detector wires to the bracket.
10. Hold the bottle cap in its normal orientation and verify there is no *Waste Bottle Full* message on the Cytometer screen.
11. Turn the bottle cap over and verify:
    a. That the float moves freely.
    b. That *Waste Bottle Full* appears on the Cytometer screen.
12. Return to step 8 in Installation Procedure above.

3.20 COLOR PRINTER UPGRADE

Purpose

Do this procedure to install the Hewlett-Packard® 1600C Color Printer, which replaces the 1200C Color Printer. Instrument model numbers affected are:

- 2356, Epics Elite ESP with Gated Amp and/or Autoclone Sorting Option and AL
- 2357, Epics Elite ESP with Gated Amp and/or Autoclone Sorting Option
- 2358, Epics Elite ESP
- 2360, Epics Elite with Gated Amp and/or Autoclone Sorting Option
- 2362, Epics Elite
- 2819, Epics Elite Analyzer

Tools/Supplies Needed

- ❑ HP 1600C Color Printer kit, PN 6914968-8, which includes:
  - HP 1600C Color Printer, PN 2016675-4
  - Parallel printer cable, PN 6027510-6
- ❑ Elite Color Printer kit, PN 6914969-3, which includes:
  - HP 1600C Color Printer kit, PN 6914968-5
  - Software driver for HP 1600C Color Printer, PN 6417268-9
  - Installation Guide for Elite Color Printer, PN 4237252-8
- ❑ HP 1600C Software Driver kit, PN 6941966-6 (for International use only), which includes:
  - Software driver for HP 1600C Color Printer, PN 6417268-9
  - Installation Guide for Elite Color Printer, PN 4237252-8
- ❑ HP 1600C Ink Cartridges kit, PN 6914967-7, which includes:
  - Magenta ink cartridge, PN 2016691-6
  - Black ink cartridge, PN 2016692-4
  - Cyan ink cartridge, PN 2016693-2
  - Yellow ink cartridge, PN 2016694-1

Printer Setup

1. Unpack the Printer and verify all items are included.
2. Setup the Printer according to the instructions supplied with the printer.
3. Install the parallel printer cables from the rear of the Printer to the parallel port at the rear of the Elite Workstation.

Software Installation

1. At the Workstation keyboard, press F2 to exit to DOS.
2. Insert the “Driver for HP 1600C” diskette into the 3.5 in. drive.
3. Type the 3.5 in. drive letter, `A` or `B`, and press Enter.

4. Type `COPY*.* C:\ELITE\GSS` and press Enter.
5. Type `C:` and press Enter.
6. Remove the diskette from the drive.
7. Verify that `C:\ELITE` is the prompt displayed.
8. Type `CD GSS` and press Enter.
9. Verify the DOS version.
10. Edit the CGI.CFG file for the HP 1600C Printer according to the DOS version instructions under either Editing CGI.CFG File: DOS 6.0 and Higher or Editing CGI.CFG File: Lower Than DOS 6.0.

Editing CGI.CFG File: DOS 6.0 and Higher

1. Type `EDIT CGI.CFG` and press Enter.
2. Locate the printer driver without a REM statement before it. This should match the printer currently in use, if any.
3. Deactivate the active printer driver:
   a. Use the keyboard cursor keys to place the cursor to the far left of the page on the same line as the driver.
   b. Type `REM` and press Spacebar.
   c. If the active printer driver is for a Laserjet, a standard dot matrix, or an Epson printer:
      1) Use the keyboard cursor keys to place the cursor to the far left of the page on the *Resolution=* line.
      2) Type `REM` and press Spacebar.
   d. If the active printer driver is for an Epson printer:
      1) Use the keyboard cursor keys to place the cursor to the far left of the page on the *Printer=Epsonx.sys* line.
      2) Type `REM` and press Spacebar.
      3) Use the keyboard cursor keys to place the cursor to the far left of the page on the *Epsonx=Prn* line.
      4) Type `REM` and press Spacebar.
4. Use the keyboard cursor keys to place the cursor to the far left of the page on a blank line and:
   a. Type `DRIVER=C:\ELITE\GSS\HPPCL5.SYS /G:PRINTER` and press .
   b. Type `HPPCL5=PRN` and press Enter.
   c. Type `RESOLUTION=300` and press Enter.
   d. Type `COLORS=16` and press Enter.
   e. Type `FF=ON`.
5. If *FF=OFF* appears without a REM statement before it:
   a. Use the keyboard cursor keys to place the cursor to the far left of the page on the *FF=OFF* line.
   b. Type `REM` and press Spacebar.

6. Verify that the CGI.CFG is setup correctly for the desired paper size:
   - For 8.5 x 11 in. paper, *PAPER=LETTER* is required. Add if necessary.

     Use the keyboard cursor keys to place the cursor to the far left of the page on a blank line and type `PAPER=LETTER.` Press Enter.
   - For A4 paper, *PAPER=ISOA4* is required. Add if necessary:

     Use the keyboard cursor keys to place the cursor to the far left of the page on a blank line and type `PAPER=ISOA4.` Press Enter.

   ATTENTION: The paper size to be used must not contain a REM statement before it.

7. Verify that the CGI.CFG file matches the following:

```
DRIVER=C:\ELITE\GSS\GSSCGI.SYS
DRIVER=C:\ELITE\GSS\META.SYS /G:OUTPUT
DRIVER=C:\ELITE\GSS\HRVGA.SYS /G:DISPLAY
DISPLAY=HRVGA.SYS
DRIVER=C:\ELITE\GSS\MSMOUSE.SYS /G:INPUT
MOUSE=MSMOUSE.SYS
MOUSEPORT=COM1
FONTS=C:\ELITE\GSS

rem standard laser printer driver
rem DRIVER=C:\ELITE\GSS\LASERJET.SYS /G:PRINTER
rem RESOLUTION=75

rem standard dot matrix printer driver
rem DRIVER=C:\ELITE\GSS\IBMGPR.SYS /G:PRINTER
rem RESOLUTION=75

rem HP Paintjet driver
rem 90 dot per inch printer driver
rem DRIVER=C:\ELITE\GSS\HPPJ90.SYS /G:PRINTER

rem Easy 2 users printer driver configuration
rem DRIVER=C:\ELITE\GSS\EPSONX.SYS /G:PRINTER
rem PRINTER=EPSONX.SYS
rem EPSONX=PRN
rem RESOLUTION=120

DRIVER=C:\ELITE\GSS\HPPCL5.SYS /G:PRINTER
HPPCL5=PRN
RESOLUTION=300
COLORS=16
FF=ON

ORIENTATION=PORTRAIT
rem PAPER=ISOA4
PAPER=LETTER
PLISTSIZE=4096
rem FF=OFF
```

Note: If paper size is A4, then the “rem” statement should appear in front of *PAPER=LETTER*, and *PAPER=ISOA4* should not contain a “rem” statement.

8. Save the changes by pressing Alt+F S.
9. Exit by pressing Alt+F X.
10. Reboot the computer by pressing Ctrl+Alt+Delete.
11. Do Printer Test.

Editing CGI.CFG File: Lower Than DOS 6.0

1. Type `EDLIN` and press Spacebar Enter.
2. Type `L` and press Enter. Eight lines followed by a series of printer configurations appears.
3. Locate the printer driver without a REM statement before it. This should match the printer currently in use, if any.
4. Deactivate the active printer driver:
    a. Type the number of the line that contains the active printer configuration and press Enter. The same statement displays above the current cursor line.
    b. Press Insert.
    c. Type `REM` and press Spacebar.
    d. Press F3 Enter.
    e. If the active printer driver is for a Laserjet, a standard dot matrix, or an Epson printer driver:
        1) Type the number of the *Resolution=* line and press Enter.
        2) Press Insert.
        3) Type `REM` and press Spacebar.
        4) Press F3 Enter.
    f. If the active printer driver is for an Epson printer:
        1) Type the number of the *Printer=Epsonx.sys* line and press Enter.
        2) Press Insert.
        3) Type `REM` and press Spacebar.
        4) Press F3 Enter.
        5) Type the number of the *Epsonx=Prn* line and press Enter.
        6) Press Insert.
        7) Type `REM` and press Spacebar.
        8) Press F3 Enter.
5. Locate a blank line and note the line number.
6. Type the line number noted and press I to insert, and press Enter.
7. Edit the CGI.CFG file:
    a. Type `DRIVER=C:\ELITE\GSS\HPPCL5.SYS /G:PRINTER` and press Enter.
    b. Type `HPPCL5=PRN` and press Enter.
    c. Type `RESOLUTION=300` and press Enter.
    d. Type `COLORS=16` and press Enter.
    e. Type `FF=ON` and press Enter.

8. If *FF=OFF* appears without a REM statement before it, add the statement:
   a. Type the number of the *FF=OFF* line and press Enter.
   b. Press Insert.
   c. Type `REM` and press Spacebar.
   d. Press F3 Enter.
9. Verify that the CGI.CFG file is setup correctly for the desired paper size:
   a. For 8.5 x 11 in. paper, *PAPER=LETTER* is required. Add if necessary:
      1) Locate a blank line and note the line number.
      2) Type the line number noted and press I to insert, and press Enter.
      3) Type `PAPER=LETTER` and press Enter.
      4) Press Ctrl+C+L then press Enter.
   b. For A4 paper, *PAPER=ISOA4* is required. Add if necessary:
      1) Locate a blank line and note the line number.
      2) Type the line number noted and press I to insert, and press Enter.
      3) Type `PAPER=ISOA4` and press Enter.
      4) Press Ctrl+C+L then press Enter.

ATTENTION: The paper size to be used must not contain a REM statement before it.

10. Press Ctrl+C+L and press Enter.
11. Verify that the CGI.CFG file matches the following:

```
1: DRIVER=C:\ELITE\GSS\GSSCGI.SYS
2: DRIVER=C:\ELITE\GSS\META.SYS /G:OUTPUT
3: DRIVER=C:\ELITE\GSS\HRVGA.SYS /G:DISPLAY
4: DISPLAY=HRVGA.SYS
5: DRIVER=C:\ELITE\GSS\MSMOUSE.SYS /G:INPUT
6: MOUSE=MSMOUSE.SYS
7: MOUSEPORT=COM1
8: FONTS=C:\ELITE\GSS
9:
10: rem standard laser printer driver
11: rem DRIVER=C:\ELITE\GSS\LASERJET.SYS /G:PRINTER
12: rem RESOLUTION=75
13:
14: rem standard dot matrix printer driver
15: rem DRIVER=C:\ELITE\GSS\IBMGPR.SYS /G:PRINTER
16: rem RESOLUTION=75
17:
18: rem HP Paintjet driver
19: rem 90 dot per inch printer driver
20: rem DRIVER=C:\ELITE\GSS\HPPJ90.SYS /G:PRINTER
21:
22: rem Easy 2 users printer driver configuration
23: rem DRIVER=C:\ELITE\GSS\EPSONX.SYS /G:PRINTER
24: rem PRINTER=EPSONX.SYS
25: rem EPSONX=PRN
26: rem RESOLUTION=120
```

```
27:
28: DRIVER=C:\ELITE\GSS\HPPCL5.SYS /G:PRINTER
29: HPPCL5=PRN
30: RESOLUTION=300
31: COLORS=16
32: FF=ON
33:
34: ORIENTATION=PORTRAIT
35: rem PAPER=ISOA4
36: PAPER=LETTER
37: PLISTSIZE=4096
38: rem FF=OFF
```

Note: If paper size is A4, then the “rem” statement should appear in front of *PAPER=LETTER*, and *PAPER=ISOA4* should not contain a “rem” statement.

12. Save the changes by pressing [E] [Enter].
13. Reboot the computer by pressing [Ctrl]+[Alt]+[Delete].

Printer Test

1. Select a histogram and press [F3].
2. Verify that the printout reflects the histogram colors displayed.

   Note: The printout may take a while to print, depending on the resolution selected.

3.21 PC MODEL PENTIUM™ 133 MHz UPGRADE

Purpose

Use this procedure to upgradselecte a workstation computer to 90 MHz Pentium for the following Elite models:

- 2356 - Elite ESP with Gated Amp and/or Autoclone Sorting Option and AL
- 2357 - Elite ESP with Gated Amp and/or Autoclone Sorting Option
- 2358 - Elite ESP
- 2360 - Elite with Gated Amp and/or Autoclone Sorting Option
- 2362 - Elite

See Table 3.21-1 for the DTX Pentium computer's technical specifications.

Table 3.21-1 Technical Specifications of the DTX Pentium Computer

Item	Specifications
Processor	Intel Pentium processor, 133 MHz
Ports	2 serial ports, async, 9 pin, 16C550 compatible, 1 parallel (EPP) port
Memory	16 megabyte standard, upgradable to 128 megabyte, 4 72 pin SIMM sockets, 70nS or faster, with parity (x36)
Cache	256k on board second level, direct mapped, write back cache
Bus	ISA
Local bus	PCI, 32 bit at 33 MHz
Hard drive	1.2 gigabyte or higher, enhanced IDE with Mode 3 timing
Controller	Tekram PCI caching controller with 4 MB cache, transfer rates up to 8 megabytes per second
Floppy drives	One 3.5 in. (1.44 megabyte), and one 5.25 in. (1.2 megabyte)
Video	SVGA with 2 megabytes standard, PCI local bus, 1024x1280 resolution/16 million colors
Expansion slots	4 ISA slots; 2 PCI slots, and 1 PCI/ISA selectable slot
Adapter card	Dual serial and one parallel ISA Adapter card provides parallel port for printer
BIOS	Flash BIOS, American Megatrends, provides PCI plug-and-play

Table 3.21-1 Technical Specifications of the DTX Pentium Computer *(Continued)*

Item	Specifications
Power supply	200 W, 115/230 V selectable, 50/60 Hz
Regulatory compliances	Safety: • US: UL 1950, 1st edition • Canada: CSA C 22.2 No. 950M-89 • Europe: TUV to EN 60950 with ZHI/618 EMI/RF: • US: FCC CFR 47 Part 15, Level B • Canada: DOC CRC c, 1374 Class B • Europe: VDE 0871 Class B, CISPR-B

Tools/Supplies Needed

- ❑ Elite Pentium Full Workstation Upgrade Kit, PN 6915005, which includes:
  - Elite DTX Pentium computer with SVGA video board and 16 MB RAM
  - Serial mouse
  - Keyboard
  - 17 in. Sony® color monitor, 1024x1280 resolution
  - Elite software library, version 4.02

OR

- ❑ Elite Pentium PC Upgrade Kit, PN 6915006, which includes:
  - Elite Intel Pentium computer with SVGA video board and 16 MB RAM
  - Serial mouse
  - Elite software library, version 4.02

Procedure

ATTENTION: Software version 4.02 is for use with Pentium computers only. Do not install version 4.02 on anything other than a Pentium computer.

1. Verify that the ac power setting on the rear of the computer matches local ac power on site.
2. Install software version 4.02 according to the README.DOC file supplied with software.
3. Remove the Lister A/B card from the old computer and install it in slot 1 of the new computer, as viewed from the front.

4. Verify the following cable connections:

   - Mouse = COM1
   - 9 pin serial cable = COM2
   - Lister cable = Connector on rear of Lister A/B card
   - Keyboard cable = To keyboard port
   - Monitor = VGA connector located on VGA card

5. Reboot the computer by pressing Ctrl+Alt+Delete.

   Note: Do not change the BIOS settings unless absolutely necessary. To change them, press Delete during the boot-up process to access the BIOS Setup Menu. The system BIOS is configured as follows:

Standard Setup

FLOPPY A	1.44 MB 3.5"
FLOPPY B	NOT INSTALLED
MASTER DISK	TYPE 1
SLAVE DISK	NOT INSTALLED

Advanced Setup

TYPEMATIC RATE PROGRAMMING	30
SYSTEM KEYBOARD	PRESENT
PRIMARY DISPLAY	VGA/EGA
MOUSE SUPPORT	DISABLED
ABOVE 1 MB MEMORY TEST	ENABLED
MEMORY TEST TICK SOUND	ENABLED
PARITY ERROR CHECK	ENABLED
HIT "DEL" MESSAGE DISPLAY	ENABLED
EXTENDED BIOS RAM AREA	0:300
WAIT FOR "F1" IF ANY AREA	ENABLED
SYSTEM BOOT UP NUM LOCK	ON
FLOPPY DRIVE SEEK AT BOOT	ENABLED
FLOPPY DRIVE SWAPPING	DISABLED
SYSTEM BOOT UP SEQUENCE	A:, C:
SYSTEM BOOT UP SPEED	HIGH
BASE MEMORY SIZE	640KB
EXTERNAL CACHE MEMORY	WR-BACK
INTERNAL CACHE MEMORY	WR-BACK
FAST POWER ON SELF TEST (POST)	ENABLED
PASSWORD CHECKING	SETUP
VIDEO SHADOW C000, 32K	CACHE
SHADOW C800, 16K	DISABLED
SHADOW CC00, 16K	DISABLED
SHADOW D000, 16K	DISABLED
SHADOW D400, 16K	DISABLED
SHADOW D800, 16K	DISABLED
SHADOW DC00, 16K	DISABLED
F000 CACHABLE	ENABLED
IRQ3	INFORMATIVE ONLY, NO SELECTION

IRQ4	INFORMATIVE ONLY, NO SELECTION
IRQ5	INFORMATIVE ONLY, NO SELECTION
IRQ7	ISA
IRQ9	ISA
IRQ10	PCI/PNP
IRQ11	ISA
IRQ12	ISA (PCI/PNP FOR OPTICAL DRIVE)
IRQ14	ISA
IRQ15	ISA

Chipset Setup

CPU TO PCI BURST WRITE	DISABLED
CPU TO PCI POSTED WRITE	ENABLED
BUS PARKING AT CPU	DISABLED
PCI MASTER BURST LENGTH	1 KB
VGA PALETTE SNOOPING	DISABLED
PCI MASTER LATENCY TIMER (CLKS)	240
NON-CACHABLE BLOCK 1	DISABLED
NON-CACHABLE BLOCK 2	DISABLED

Power Management Disabled Peripheral Setup

ON BOARD FLOPPY CONTROLLER	ENABLED
ON BOARD PRIMARY/SECONDARY IDE	DISABLED
PCI IDE CARD PRESENT IN	ABSENT
SERIAL PORT 1	3F8H
SERIAL PORT 1 FIFO	DISABLED
SERIAL PORT 2	2F8H
SERIAL PORT 2 FIFO	DISABLED
PARALLEL PORT	278H

3.22 ELITE 256 SORT UPGRADE

Purpose

Use this procedure to install and set up the Elite 256 SORT Upgrade kit.

Tools/Supplies Needed

- ❑ Elite 256 Sort Upgrade kit, PN 6915082, which contains:
  - Bitmap and Sort Decision R2 card, PN 6706232
  - Elite version 4.5 Software, PN 6915099
  - Elite ESP Reference Manual, PN 4235904

Procedure

Install the New Bitmap and Sort Decision Card

1. At the electronics pedestal, remove the right panel to gain access to the data acquisition electronics.
   a. Remove the four Phillips-head screws securing the panel.
   b. Disconnect the grounding cable from the Cytometer chassis.
   c. Set the panel aside.
2. Locate the Bitmap and Sort Decision card in slot 12 of the lower card cage.
   a. Remove the ribbon cable attached to the card.
   b. Remove the circuit card.
   c. Set it aside.
3. Install the new Bitmap and Sort Decision R2 card in the slot in slot 12.
4. Reconnect the ribbon cable so that the arrow on the connector is pointing up when the cable is attached.
5. Reinstall the right panel on the electronic pedestal.
   a. Reconnect the grounding cable from the Cytometer chassis.
   b. Secure the panel using the four Phillips-head screws removed earlier.

Install The Elite Software

1. Locate the Elite version 4.5 Software disk 1 of 2.
2. Insert disk 1 into the 3.5-inch floppy drive.

   Note: If the Workstation has two floppy drives, the 3.5-inch drive is designated as the B: drive. If the 3.5-inch drive is the only floppy drive, the drive is the A: drive.
3. If the Workstation is presently in the Elite Software, press [F2] then [Enter] to exit to DOS.
4. At the C:\ELITE prompt, type `A:` then press [Enter].
5. To access the README document:
   - If the Cytometer does not have a printer, go to step 6.
   - If the Cytometer is equipped with a printer, go to step 7.

6. If the Cytometer does not have a printer,
   a. At the A: prompt (B: prompt if the system contains two floppy drives), type `README.DOC|MORE` then press Enter.
   b. Review the readme.doc file before installing the Elite Software.
   c. Go to step 8
7. If the Cytometer is equipped with a printer:
   a. At the A: prompt (B: prompt if the system contains two floppy drives), type `PRINT README.DOC` then press Enter.
   b. Review the printed readme.doc file before installing the Elite Software.
8. At the A: prompt (B: prompt if the system contains 2 floppy drives):
   a. Type `INSTALL` then Enter.
   b. Follow the instructions on the screen to install the Elite Software.
9. When the installation of the Elite Software is complete:
   a. From the Main screen, select **File ▸▸ Open**.
   a. Select a protocol then click on **Ok**.
10. Press F9 then Enter.
11. When the error message, *UNABLE TO RECEIVE CYTOMETER PROGRAM VERSION, PLEASE ACKNOWLEDGE* appears, press Enter again.
12. When the message *DO YOU WISH TO TRANSFER THE CONTROL FILE?* appears, press Y then Enter.
13. On the Cytometer right monitor, the number “1” appears. Wait for this number to sequence from 1 to approximately 425. This process will take several minutes.
14. If the Elite is equipped with the Autoclone sorting option:
    a. When the Control File is transferred, the message *PLEASE POWER UP THE VALVES AND CALIBRATE THE AUTOCLONE* appears on the Cytometer screen.
    b. Make sure this process is completed before continuing.
15. Press F10 to stop acquisition.
16. At the Menu bar, select **Applications ▸▸ Utilities**. The Utilities menu lists a series of options in one column and whether or not these options are "PRESENT" in a second column.
17. Locate the **Sort 256 x 256** option and verify the word “PRESENT” is associated with the Sort 256 x 256 option. If this option is listed as PRESENT, the 256 Sort upgrade is successfully installed.
18. Under Heading 5.1, VERIFICATION INSPECTION PROCEDURE,
    a. Complete the instructions under heading Operational Test - Cytometer Electronics, Optics, or Fluidics as written.
    b. Complete the instructions under heading Operational Test - Sorting Ability as written.

ILLUSTRATIONS

TABLES

4.1 OPTICAL ALIGNMENT PROCEDURE

System Alignment

Purpose

The alignment procedures depend on the types of lasers installed and their optical configuration. This procedure applies to the standard configuration, which includes the air-cooled Argon and HeNe co-linear beam configuration.

Refer to the appropriate procedure for other laser configurations:

- EPICS Elite Options Manual:
  - HeCd Model 56 alignment
  - HeCd Model 74 alignment
  - Innova 305 alignment
  - Innova 90-5 alignment
- Heading 3.7, OPTIONAL LASER INSTALLATION contains procedures for aligning HeCd, Innova 305, Innova 90 and HeCd lasers.
- Heading 3.14, ENTERPRISE LASER UPGRADE contains procedures for Enterprise laser alignment.
- Heading 3.9, GATED AMPLIFIER UPGRADE contains procedures for alignment for Gated Amp operation.

WARNING Risk of personal injury due to exposure to Laser beam. Do not proceed until you review, understand, and adhere to the safety precautions for lasers under Heading 1.2.

Standard Configuration Procedure

Tools/Supplies Needed

❑ None.

Procedure

1. Install the two short targets on the optical plate and verify alignment of the lasers. Do not align the lasers unless needed. See Standard Laser Alignment.
2. Verify back reflection. Adjust flow cell as necessary using Flow Cell Alignment, Initial.
3. Refer to the Headings 3.14 and 3.15 in the Elite ESP Special Procedures and Troubleshooting manual to:
   a. Remove the targets, reinstall the scatter sensor, and use the camera to align the flow cell.
   b. Use fluorospheres to align the beam shaping lens, focus the laser and the fluorescence detection lens.
   c. Optimize adjustments for best CVs and signal amplitudes.

Standard Laser Alignment

Purpose

This procedure describes alignment of the standard air-cooled Argon and HeNe lasers on the optical table for co-linear beams with no other lasers present. Refer to the correct laser alignment procedure in this section for all other laser configurations or gated amp alignment.

Note: As a general alignment principle, adjust the front laser mount to align to the target closest to the laser. Adjust the rear laser mount to align to the target furthest from the laser. A series of small, iterative adjustments works best.

Tools/Supplies Needed

- ❑ None.

Procedure

1. Mount the tall targets in front of the HeNe laser.
2. Remove the HeNe dichroic, if present.
3. Turn ON the HeNe laser.
4. Adjust the HeNe mounting to align the beam through the tall targets:
   a. Use the center holes on the three hole targets to align the beam.
   b. Adjust the HeNe by moving the laser feet for horizontal movement. Adjust vertical movement by loosening the lower screw on the laser foot, adjusting the upper screw to move the laser. Retighten the lower screw.
5. Remove the tall targets and install the short targets in place of the scatter sensor and next to the laser shutter.
6. Install the HeNe dichroic in front of the HeNe.
7. Adjust the dichroic to align the beam through the targets. If the beam passes through the target closest to the laser but does not align with the target in the scatter sensor position the output end of the HeNe laser needs a small adjustment. Work with the HeNe adjustment and the dichroic until the beam passes cleanly through both targets.
   a. Turn HeNe laser off.
   b. Turn Argon laser on.
8. Align the Argon laser beam through the targets:
   a. Adjust the Argon laser mounts to align the laser beam through the targets.
   b. Make vertical adjustments by loosening the upper nut on the mounting post and adjusting the lower nuts, then re-tightening the upper nuts.
   c. Make horizontal adjustments by loosening the screws securing the laser to the base and sliding the laser, then retightening.

   Note: As a general alignment principle, adjust the front laser mount to align to the target closest to the laser. Adjust the rear laser mount to align to the target furthest from the laser. A series of small, iterative adjustments works best.

Flow Cell Alignment, Initial

Use this procedure to center the flow cell channel in the laser beam. The flow cell face must be normal (perpendicular) to the laser beam.

1. Remove the beam shaper.
2. Verify that the laser beam is centered on the scatter sensor. If not, or if there is any doubt as to laser alignment, install the targets align the laser.
3. Turn Sheath on and open the laser shutter.
4. Adjust the flow cell vertically to ensure the laser beam is passing through the quartz cell.
5. Rotate the flow cell so the laser beam reflects back through the laser:
   a. Loosen the nut securing the flow cell to the bimorph.
   b. Rotate the flow cell as needed.
   c. Tighten the nut.
6. Adjust the flow cell tilt so the laser beam reflects back through the laser shutter:
   a. Loosen the two bolts securing the flow cell stage to the vertical plate only enough to allow the flow cell to be tilted.
   b. Adjust the flow cell tilt as needed.
   c. Tighten the bolts.
7. For systems with the flat bimorph:
   a. Slightly loosen the two bolts which secure the bimorph plates to the flow cell stage.
   b. Slide the plate and flow cell assembly in order to center the laser beam on the flow channel. Do not rotate the flow cell in this step.
   c. Tighten bolts.
8. For systems with the newer enclosed bimorph, adjust the thumbscrew to center the laser beam on the flow channel.
9. Observe reflected laser beam and path of beam through the flow cell. Repeat above adjustments until reflections are orthogonal and beam is centered on channel.

Beam Translator-Equipped System Alignment

Purpose

Beam translators are available as upgrade parts. They are not part of the modification kit for the HeCd laser but are offered as a sales option.

This translator is used to adjust the beam to the proper height. The resultant beam is parallel to the exit beam of the laser. This allows the user to use either co-lateral or separated beams depending on the application.

Tools/Supplies Needed

- ❑ Beam Translator Kit, PN 6912985-4, which includes:
  - Beam translators, one for each laser:
  - UV light (either HeCd or Argon UV laser), PN 6858244-0
  - 488 nm light (Argon laser), PN 6858245-8

  - 633 nm light (HeNe laser), PN 6858243-1
  - 3 micro optical rails for mounting, PN 3814206-2
  - 2 machine screws for each of above, PN 2851763-7
- ❑ New laser alignment targets with three target holes
  - Large targets, PN 6857274-6
  - Small targets, PN 6857236-3
  - Mounting screws, PN 2851920-6
- ❑ Fluorospheres

Laser Mounting Procedure

Note: The long dimension of the optical bench is referred to as the “columns”. The shorter dimension of the optical bench is referred to as the “rows”.

1. Mount the UV laser in the farthest rear position possible on the table top with room for adjustment of the laser X-Y axis, column 16, row 9.

   Note: You may want to mount the HeCd lasers closer to the flow cell.
2. Parallel with the long dimension of the optical bench, leave room in front of the laser output for the beam expander/reducer, a target, and the optical rail.
3. Mount the Argon laser in the column 8, 9, and 10, row 7.

   Note: Leave room in front of the laser output for the beam expander/reducer, a target, and the optical rail.
4. Mount the HeNe laser in its usual position next to the fluorescence collection assembly.

   Note: Leave room for alignment of the X-Y axis of the HeNe laser between it and the fluorescent collection assembly. Leave room for a target, a beam expander/reducer, and the optical rail.

Laser Alignment for Gated Amp Systems (Standard Laser Alignment)

1. Decide which laser needs to be used as a trigger source for the Gated Amp.

   Note: This needs to be the laser the cell hits first).
2. Decide which lasers are to be used and in what time/space dimension, top beam or bottom beam.

   Note: For this alignment procedure, we will align the Argon on top, and the HeNe and UV lasers on the bottom.
3. Install the two standard 5 in. targets on the optical bench in front of the HeNe laser separated by two mounting holes.

   Note: Leave enough room for yourself for a good view of the alignment holes in the targets.
4. Remove these components:
   a. Scatter sensor
   b. Laser shutter
   c. Beam shaper.
5. Raise the flow cell up as far as possible using the flow cell vertical adjustment knob.

6. Install the two smaller laser alignment targets on the vertical optical plate.
7. At the Cytometer Control screen:
    a. Turn the HeNe laser on.
    b. Turn the air-cooled Argon lasers on.
8. Turn the UV laser on using lasers own on/off switch.
9. Close the air- cooled Argon and the UV laser output shutters to prevent any beam over-exposure accidents.
10. With only the HeNe emitting, align the HeNe using the Standard Alignment Laser procedure first through the two targets on the optical bench, then through the two vertical optical plate-mounted-targets using the 90 degree dichroic.
11. Close the shutter on the HeNe laser.
12. Move the two optical bench-mounted Targets in front of the air-cooled Argon laser.
13. Open the shutter on the air-cooled Argon aser.
14. Align the Argon Laser using the Standard Alignment Laser procedure for the two 5 in. targets on the optical bench, then for the vertical optical plate-mounted targets using the 90 degree dichroic.
15. Close the shutter on the air-cooled Argon laser.
16. Move the two optical bench-mounted 5 in. targets in front of the UV laser.

WARNING Risk of personal injury from laser beam exposure. Wear a pair of latex surgical gloves to reduce the amount of UV in the event you accidentally place your hands in front of the beam. Remove all jewelry (rings, watches, etc.) and be careful of tools that could reflect the beam.

17. Open the laser output shutter on the UV laser.
18. Align the UV laser using the Standard Laser Alignment procedure for two optical bench-mounted 5 in. targets, then for the two smaller vertical optical plate-mounted targets using the 90 degree full mirror.

    Note: Use a business card or fluorescent “day glow” paper to help detect position of the UV laser position.
19. Close the laser output shutter on the UV laser.
20. Place the “screw-on” targets (input and output lenses) on the beam reducer.

    Note: The old style beam reducer can be used, but it does not have built-in targets.
21. Install the beam reducer on the optical bench as close as possible to the output of the UV laser.

    Note: Leave enough room to remove the “screw-on” targets from the beam reducer when done.
22. Open the UV laser output shutter and align the X-Y-Z axis of the beam reducer for optimum alignment through the two optical bench-mounted targets and the two vertical optical plate-mounted targets.
23. Remove the “screw-on” targets from the beam reducer when you are satisfied with the alignment of the beam reducer.

IMPORTANT Risk of misalignment if both the Collinear Laser Alignment and Gated Amp Laser Alignment are used on an instrument. Select the appropriate procedure. Do not do both the Collinear Laser Alignment Fine Tuning for Three Lasers and Gated Amp Laser Alignment (Time/Space Separated Laser Alignment) on an instrument.

24. Do **one** of the following:
    - Collinear Laser Alignment Fine Tuning for Three Lasers, or
    - Gated Amp Laser Alignment (Time/Space Separated Laser Alignment).

Collinear Laser Alignment Fine Tuning for Three Lasers

1. Remove the targets:
    a. Remove the two standard 5 in. optical bench-mounted laser alignment targets.
    b. Remove the two standard vertical optical plate-mounted targets.
2. Install the following:
    a. Scatter sensor assembly
    b. Beam shaping assembly
    c. Laser shutter.
3. Lower the flow cell to its proper position using the “flow cell Y” vertical adjustment knob and the camera.
4. Remove optical filters except the 1.0 ND filter from the forward scatter sensor filter holder, and install the filter holder back in the FALS sensor.

    Note: Remove the 525 BP filter to obtain a side scatter/fluorescent signal in PMT2.
5. Press **SHEATH** to begin sheath flow.
6. At the Cytometer’s Scope screen:
    a. Select **Fals Peak** for the lower trace.
    b. Select **PMT 2 Peak** for upper trace.

    Note: Throughout this procedure, Fals Peak and PMT 2 are used for alignment purposes.
7. Open the HeNe laser output shutter and run fluorospheres at a data rate of approximately 100.
8. Align the optical area for maximum signal on both traces of Digiscope using the standard approach:
    a. Flow cell X/Y adjustment
    b. Beam shaper X/Y/Z adjustment
    c. Fluorescence collection assembly focus (snout).
9. Align the Argon laser:
    a. Open the Argon laser output shutter.
    b. Align for maximum collinear signals (both Argon and HeNe signals appear to be on top of each other) on both traces of Digiscope using Argon laser 90 degree dichroic adjustments.
    c. Close the Argon laser output shutter.

ATTENTION: Do not change any flow cell or beam shaper alignment because you will undo the alignment that you just did for the HeNe.

10. Align the UV laser:
    a. Open the UV laser output shutter.
    b. Align for maximum collinear signals on both traces of the Digiscope using the 90 degree mirror only.

ATTENTION: Do not change any flow cell or beam shaper alignment because you will undo the alignment that you just did for the HeNe.

11. Open the Argon laser output shutter.
12. Now that all three lasers are active, verify that all three resultant signals occur in the same time-frame (collinear, all three appearing to be on top of each other):
    a. Block each laser off one at a time with a target or an opaque object.
    b. Observe the change in displayed signals on the Digiscope.

Gated Amp Laser Alignment (Time/Space Separated Laser Alignment)

1. Close the Argon laser output shutter.
2. Set the UV laser power up/or down to create a distinguishable difference in amplitude between the HeNe and UV laser resultant signals.
3. Install the adjustable beam translator between the HeNe laser and its adjustable dichroic, leaving space between the translator and the optical rail to mount a single laser target.
4. Verify the following:
    a. The translator knobs face away from the laser.
    b. The translator window is perpendicular to the optical bench.
5. Adjust the translator height in post for the laser to penetrate as close to the center of the translator window as possible.
6. Refer to the multi-hole target and set the alignment mode to Top (1). Move the pin on the bottom of the target to line up with desired target hole, and lock pin down with the set screw.
7. Install the middle target in the space you left between the beam translator and the HeNe dichroic, ensuring the target is square with the table top.
8. Rotate the translator window so the top of the HeNe beam passes through the middle of the top target hole.
9. Remove the HeNe laser target.

10. Adjust between the peak of the HeNe laser-generated signal and the peak of the UV laser-generated signal by using the adjustable HeNe laser dichroic on the optical rail. Look at the lower trace on the Digiscope Screen and:
    - Adjust for 20 microseconds for a 100 μ quartz tip.
    - Adjust for 30 microseconds for a 76 μ quartz tip.
    - Adjust for 3.5 microseconds for a 76 μ jet-in-air tip.

    Note: The UV signal is on the far left on the Digiscope screen. Test this by increasing/decreasing the UV laser power and observing the far left peak.
11. Close the HeNe laser output shutter.
12. Install the adjustable beam translator between the Argon laser and its adjustable dichroic, leaving room between the translator and the optical rail to mount a single laser target.
13. Verify the following:
    a. The translator knobs face away from laser.
    b. The translator window is perpendicular to the optical bench.
14. Adjust the translator height in the post for the laser to penetrate as close to the center of the translator window as possible.
15. Refer to the multi-hole target and set the alignment mode for "lowest" target hole. Move pin on bottom of target to line up with desired target hole, and lock the pin down with the setscrew.
16. Install the lowest target in the space you left between the beam translator and the Argon dichroic, ensuring the lowered target is square with the table top in all respects.
17. Rotate the translator window so the Argon beam is aligned with the lowest laser target hole.
18. Remove the lowered Argon laser target.
19. Adjust between the peak of the Argon laser-generated signal and the peak of the UV laser-generated signal by using the adjustable Argon dichroic on the optical rail. Look at both traces on the Digiscope screen and:
    - Adjust for 20 microseconds for a 100 μ tip
    - Adjust for 30 microseconds for a 76 μ tip
    - Adjust for 3.5 microseconds for a 76 μ jet-in-air tip.

    Note: The peak of the UV laser-generated signal is the far middle one of the Digiscope screen. Test this by increasing/decreasing the UV laser power and observing the middle peak.
20. Refer to the multi-hole target and set the alignment mode for highest hole. Move the pin on the bottom of the target to line up with desired target hole, and lock the pin down with setscrew.
21. Install the highest target in the space you left between the beam translator and the UV dichroic on the optical rail, ensuring the target is square to the table top in all respects.
22. Rotate the translator window so the UV beam aligns with the top hole in the alignment target.
23. Remove the raised UV laser target.

24. Use the adjustable UV laser dichroic on the optical rail and look at both traces on the Digiscope screen. Adjust the UV beam using the 90 degree UV dichroic only for the same time-frame as the HeNe laser-generated signal.
25. Verify that the time separation between the peak of the UV and HeNe laser-generated signals and the Argon laser-generated signals is:
    - 40 microseconds for the 100 µ sort sense tip at 12 psi
    - 60 microseconds for the 76 µ sort sense tip at 12psi
    - 7 microseconds for the 76 µ jet-in-air tip.

    Note: With the Argon laser-generated signal occurring first, it is on the far left of the Digiscope screen. Test this by increasing/decreasing the UV laser power and observing the far right peak; block the HeNe laser and observe the far right peak; and raise/lower the Argon laser power and observe the far left signal.
26. Verify correct alignment:
    - The Argon laser should exit near the center of the beam shaper
    - The UV laser should exit the beam shaper slightly below the Argon.

    They should remain parallel through the flow cell all the way to the Fals sensor. Verify this by placing a business card in the beams in several different places. Lower laser power is easiest on the eyes. It is recommended to place the card immediately after the beam shaper just before the scatter sensor.

Camera Adjustment Procedure

The camera assembly must be properly adjusted to obtain the best image for alignment and sorting. This procedure describes how to correctly adjust the camera to achieve the following conditions:

- Stream centered in the illuminated (strobe) area.
- Strobe illumination centered on monitor.
- Stream and strobe illumination remain centered at all zoom settings.
- Stream to remain in focus at all zoom settings.
- When camera position is all the way up, the flow cell alignment image will remain centered on the monitor at all zoom settings.
- When zoom is in full-out position, zoom setting is at the mechanical minimum setting, which is 1.0 for newer camera assemblies, and 0.7 for older camera assemblies.
- Zoom or position mechanisms should not bind.

Aiming the Camera

1. Optimize the prism mounted on the scatter sensor:
    a. Loosen the securing setscrew.
    b. Rotate the adjusting lever to center the stream image in the strobe.
2. Optimize the periscope:
    a. Loosen the radiator hose clamp (older units) or the setscrews securing the periscope to the base (newer units).
    b. Rotate the periscope.

3. Repeat steps 1 and 2 as needed until:
   - The stream is centered in the illuminated (strobe) area.
   - The strobe illumination is centered on monitor.
   - The stream and strobe illumination remain centered at all zoom settings.

Focusing the Camera

The primary focus adjustment for the camera assembly is the upper internal mirror on the periscope. Focus the camera according to the following procedure.

1. Loosen the securing setscrew at the upper internal mirror on the periscope.
2. Move the mirror vertically until the best focus is achieved, and tighten setscrew.

   Note: This adjustment affects the camera's position.
3. Vary the zoom and ensure that the stream remains focused. If the stream does not remain focused, do step 4.
4. If the stream does not remain focused:
   a. Loosen the setscrew that secures the camera to the horizontal tube, and slightly move the camera.

      Note: This step requires you to readjust the primary focus as instructed in step 2.

   b. Make small adjustments until optimal zoom focus is achieved.

      Note: Optimize the focus at a high zoom setting so the droplets can be clearly seen for sorting. Minimal loss of focus at extreme zoom settings is acceptable.

Zooming the Camera

1. Zoom out as far as possible to get the smallest image.
2. Verify that the zoom number in the periscope window is 1; adjust if necessary.

   Note: Older cameras with the hose clamp secured periscope may go to 0.7, which is acceptable.

ATTENTION: Do not lose the screws. Do not let the belt fall off.

3. Adjust the zoom:
   a. Loosen the screws shown in Figure 4.1-1 to release the tension from the pulley.

   b. Without rotating the pulley, rotate the zoom to the 1 (or 0.7 position).

   c. Tighten (or replace) the belt and the screws.

   OR

   a. Carefully loosen the setscrew that secures the belt pulley to the feedback pot.

   b. Use a short screwdriver to move the pot shaft inside the pulley.

      Note: The pot, motor, and control card form a closed loop. Therefore, if you move the pot, the motor runs continuously to attempt to re-zero the pot. Do not allow the motor to run past the limits of the zoom mechanism. The proper adjustment technique is to turn the pot shaft slightly to let the motor move in small increments.

   c. Tighten the setscrew.

Positioning Movement of the Camera

When camera position is all the way up, the flow cell alignment image must remain centered on the monitor at all zoom settings. If the image does not remain centered, do this procedure. See Figure 4.1-1.

1. Move the camera up until it stops.

ATTENTION: Do not lose the setscrew. Do not allow the belt to fall off.

2. Loosen the screws holding the position pot pulley and move (do not rotate) the pulley to release tension on the belt.
3. Rotate the pulley slightly to move the camera to a new position and stop the movement by returning the pulley to its original position. Repeat this step until the image is correct.
4. Move the pulley to tighten the belt and tighten the screws.

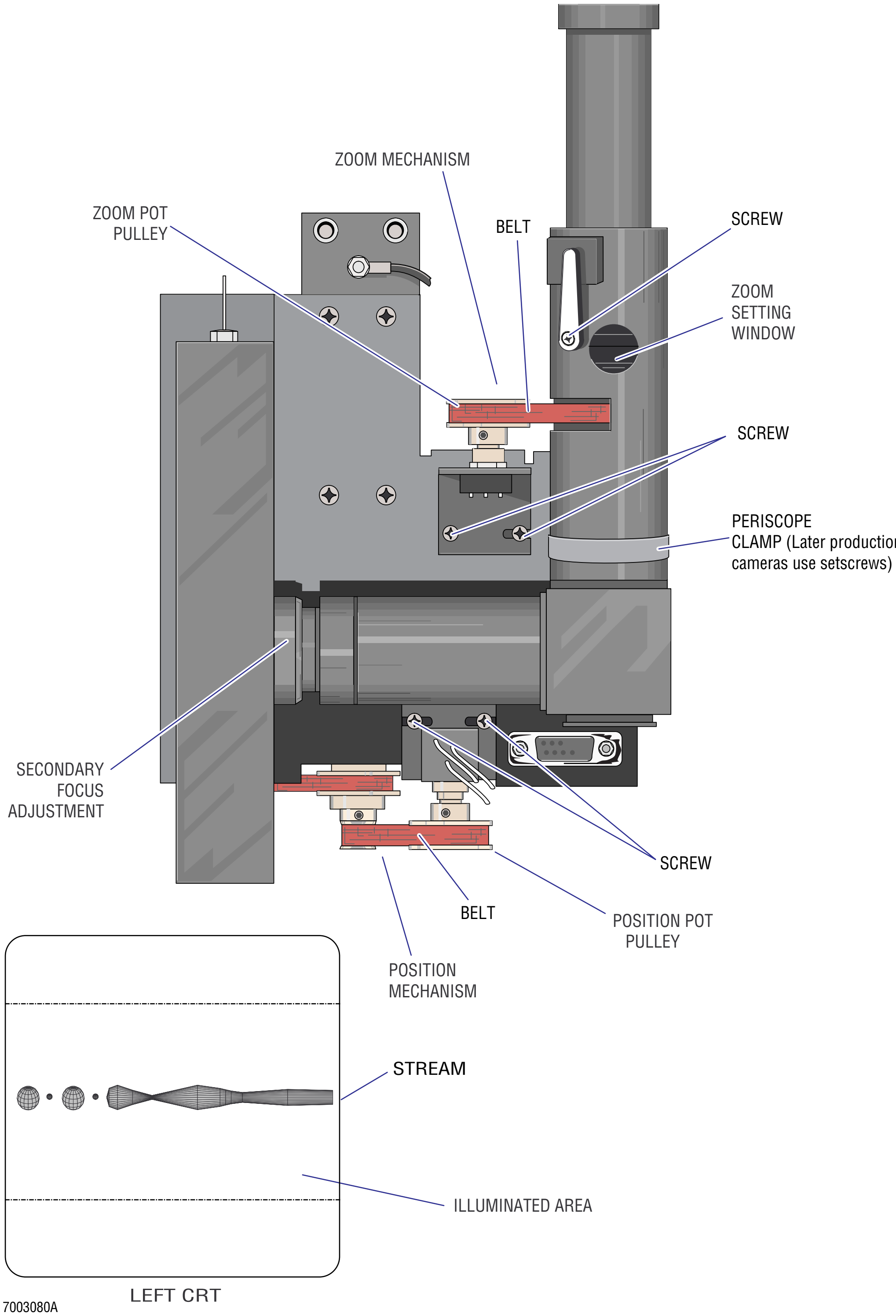
Optimizing the Image

Adjust the camera video level for best image definition as follows:

1. Remove the left front panel from electronics pedestal.
2. Use the camera to observe sheath flow.
3. Adjust R99 on the Camera Interface card to obtain the best camera image.

   Note: This control is a video gain adjust that controls the brightness of the image. Increase the brightness until the image begins to bloom or saturate. Decrease the brightness until good image quality is observed.

Figure 4.1-1 Camera Assembly Adjustment



4.2 SORT WAVEFORM VERIFICATION AND ADJUSTMENT PROCEDURE

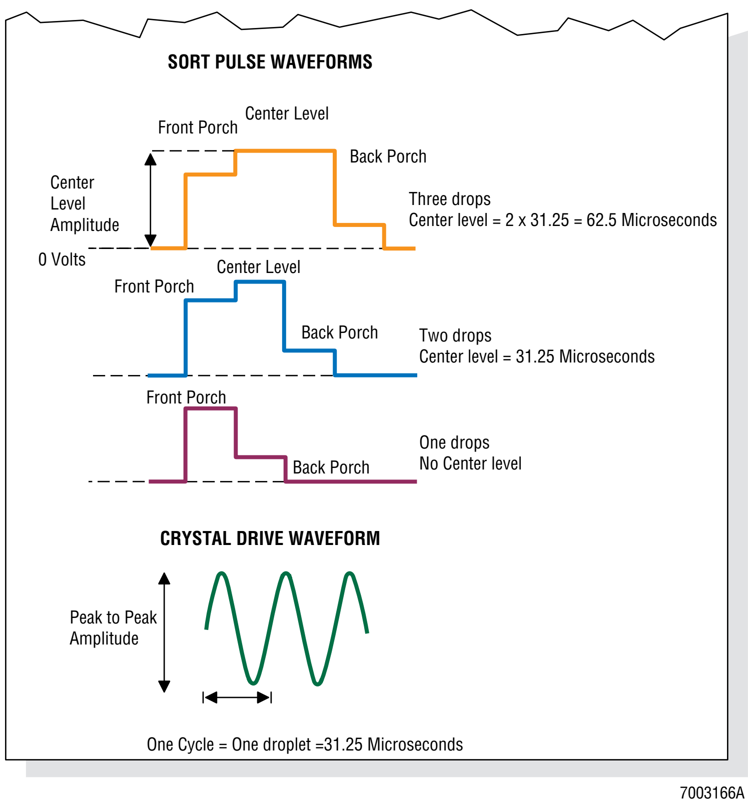
Purpose

This procedure describes how to verify and adjust the Crystal Drive and Sort Pulse signals. Perform this procedure when any of the following conditions occur:

- System installation
- Replacement of Sort Oscillator card, Sort Output card, ±90 V power supply, 36 V power supply or sort transistor
- Sort problems.

See Figure 4.2-1.

Figure 4.2-1 Waveforms



Regarding Figure 4.2-1:

- Set **Drive Frequency** to 32 kHz. At this frequency, each droplet period will be 31.25 microseconds.
- Set the **Crystal Drive** to 100%.
- Set the **Deflection** amplitude to 100%.
- Set **Sort Test**, **Sort Right Enable**, **Sort Left Enable** ON.
- Set the **Stream** control to sort pulse baseline of 0 V.

Verify the following with an oscilloscope:

- Front porch length = Back porch length = 31.25 microseconds.
- Center level length = Number of droplets to sort = 1.
  See text for voltage specifications from baseline to center level.
- Front and back porches adjust from 0 to 100% of center level.

Tools/Supplies Needed

- ❑ Oscilloscope
- ❑ DVM

Crystal Drive Test Procedure

1. Monitor the signal with a scope at the flow cell where the cable connects to the Bimorph assembly.
2. Set **Drive Frequency** to 32 kHz.
3. Set **Crystal Drive** to 100%.
4. Ensure that the wave form is sinusoidal and measures 160 V peak to peak ±5 V with little distortion.
   - If the voltage is incorrect, do Crystal Drive Adjustment Procedure.
   - If voltage is correct, go to step 5.
5. Decrease **Crystal Drive** setting and ensure the signal reduces proportionately.

Crystal Drive Adjustment Procedure

1. Verify that the **Crystal Drive** is at 100% and the **Drive Frequency** is at 32 kHz.
2. Connect the oscilloscope to the Sort Output card, TP1.
3. Adjust R84 on the Sort Oscillator card for 8.5 V peak to peak.
4. Connect the oscilloscope to the bimorph cable at the flow cell.
5. Adjust R118 on the Sort Output card for 160 V ± 5 V peak to peak signal.

Sort Pulse Amplitude Test

1. Set **Drive Frequency** to 32 kHz.
2. Connect the oscilloscope to the alligator clip that attaches to the flow cell's sample insertion rod.

   Note: You can leave the clip connected to the flow cell.
3. Turn on:
   a. **Sort Test**
   b. **Sort Left Enable**.
   c. **Sort Right Enable**.
4. Set **Sort Right** and **Left Stop** counters to OFF.
5. Press **START** and verify the sort counters are counting the Sort Test pulses.
6. Set **Deflection** to 100%.

7. Set **Drops Sorted** to 3.
8. Adjust **Stream** offset so the signal baseline is 0 Vdc.
9. Measure amplitude of the center level from the baseline and verify the value is 80 V ±2.5 V.
   - If the value is incorrect, do Sort Pulse Amplitude Adjustment.
   - If the value is correct, go to step 10.
10. Verify that the sort pulse appears as shown in Figure 4.2-1 for 1, 2, and 3 drop sorts.
11. Verify that the negative-going pulse is symmetrical with positive-going pulse.
12. Adjust front and back porches to ensure they can be varied from zero to 100%.
13. Return front and back porches to reference values:
    - Front porch at 80%
    - Back porch at 20%.

Sort Pulse Amplitude Adjustment

1. Set **Drive Frequency** to 32 kHz.
2. Turn on **Sort Test**.
3. Turn on **Sort Left Enable.**
4. Turn on **Sort Right Enable**.
5. Set **Sort Right** and **Left Stop** counters to OFF.
6. Press **START** and verify the sort counters are counting the **Sort Test** pulses.
7. Set **Deflection** to 100%.
8. Set **Drops Sorted** to 3.
9. Adjust **Stream** offset so the signal baseline is 0 Vdc.
10. Connect the oscilloscope to the Sort Output card, TP2.
11. Adjust R94 on the Sort Oscillator card for a baseline-to-center level value of 4.0 ±0.1 V.
12. Connect the oscilloscope to the flow cell sample insertion rod.
13. Adjust R119 on the Sort Output card for a baseline-to-center level value of 80 ±1 V.

Phase Settings

1. Connect one scope channel to the crystal drive and the other to the sort pulses.
2. Trigger on the sort pulse channel.
3. Vary the phase and verify a corresponding shift in the crystal drive with respect to the sort pulses.

   Note: Each phase increment (0.0 to 0.9) equals a one-tenth wavelength shift of the crystal drive sinewave signal.

Delay Settings

1. Acquire beads and set up the windows necessary to obtain sort pulses.
2. Turn **Sort Test** OFF.

3. Sort all the beads to the right that are in the GFL histogram:
   a. Select **GFL** for the lower Digiscope trace.
   b. Leave one scope channel connected to the insertion rod and connect the other channel to the GFL test point on the lower trace (LT) BNC connector on the Aux power panel.
   c. Be sure you can see both pulses on the scope, then set the trigger to Sync on the GFL pulse. The sort pulse displayed on the scope is delayed by the Delay entered on the touch screen.
4. Verify that the delay observed varies as the delay is varied on the touch screen.

   Note: Jitter of less than or equal to 1 drop is expected in the sort pulse timing. This is normal since the sort pulses are synchronous with the crystal drive.

Sync Adjustment

1. Connect one scope channel to the crystal drive and the other to the sort pulses.
2. Sync the scope on the sort pulses.
3. Verify that, as in the sort test, that the sort pulses are synchronous with the crystal drive.

Sort Function Verification

IMPORTANT Risk of compromising purity and recovery if the sort verification procedures are not strictly followed. To obtain a stable, successful sort, carefully follow each sort verification procedure.

1. If instrument has been shutdown:
   a. Prepare and start up instrument for daily operation as instructed in the Operator's Guide.
   b. Check for proper alignment and flow.
   c. Perform any cleaning or adjustments at this point.
2. If saline is evident, clean the deflection plates.
3. Plug in defection plates by placing the top of the strobe between the bottom of the beam shaping lens and the bottom of the lens holder.
4. Center the stream within the sort plate opening by adjusting the knob on the deflection body holder to center the ground plate opening around the stream.

IMPORTANT Risk of misalignment. Any movement of the Z-axis of the flow cell stage can affect all other optical alignments. This knob should only require adjustment once, and that is when a new flow cell, flow body, or bimorph stage is replaced.

5. If the stream is still too close to one side or the other, verify through proper alignment procedures that the Z-axis of the flow cell stage is adjusted correctly. Any movement of this axis can affect the rest of the optical alignment.

Drive Frequency Sort Settings

1. Set the **Drive Frequency** at 32 kHz.
2. Set the **Crystal Drive** to 75%.

3. While viewing the droplets above the ground plate, scan through the frequency settings using the tenth decimal adjustment.

   Note: It is best to start at 32 kHz and scan down.

4. Stop scanning when the highest breakoff (closest to the flow cell) is achieved and the droplets appear very focused and sharp.
5. Set a cursor tangent to the bottom of the last attached drop.
   - If the last attached droplet is not yet visible above the top of the ground plate, increase the **Crystal Drive** from 75% to 85%.
   - If the last attached drop is still not visible, move the deflection body down slightly.
   - Adjust the drive for a thin filament connecting the last attached drop.
6. Press **DEBUBBLE** twice with **High Voltage** off and ensure that the breakoff returns to the same place.

   If the breakoff does not return to the same place, the flow cell has a bubble or a partial clog. Remedy this before continuing. In extreme cases, you may have to remove or replace the flow cell.

7. Move the camera all the way down stream and look at the droplets to verify they are clear, sharp and fairly round in shape without any satellite droplets.
   - If the droplets are acceptable, go to step 8.
   - If the droplets are not acceptable, slightly adjust the frequency using the decimal setting to improve the shape and clarity of the drops.
8. Move the camera back upstream and ensure that the last attached droplet position has not changed significantly (by more than 3 drops).

   Note: You may need to adjust the sort deflection body slightly to view the last attached drop. It is helpful if it is visible just above the ground plate so that it can be easily monitored.

Phase Adjustment

1. Set **Sort Counters** to OFF.
2. Turn on the **Sort Test**.
3. Turn on the **Left Sort** and **Right Sort**.
4. Turn on the **High Voltage**.
5. Set **Sort Droplet** to 1 and press **START**. Sort counters should be running.
6. Increase the deflection by increasing the **Deflection** amplitude to bring out the side streams enough, but do not allow them to hit the sort plates (about 90% on the standard sort plates).
7. Use the phase adjustment (the decimal portion of the delay setting) to find the best side streams. For the best side streams:
   a. Find the worst phase setting and subtract 0.5.
   b. Set the phase to that number for the best side streams.
8. You should get at least seven of the phase settings to give good, clean side streams. If not, begin again at step 1.

9. Check the side streams with a sample running:
   a. Turn off **Sort Test**.
   b. Load your sample protocol.
   c. Set **Sort Setting** to **Receive**.
   d. Set **Cytometer Settings** to **Send**.

IMPORTANT Risk of compromising sort stability sample flow if sample flow is adjusted more than 10 units above the optimal setting for running beads. Avoid compromising sort stability by not adjusting the sample flow more than 10 units above the optimal setting for running beads.

   e. Change the **Sort Settings** to **Send** to send your sample sort gates.
      - Run the actual sample at the data rate desired for sorting. Note the differential pressure required to achieve the desired sort rate and use this same Diff pressure when doing the sort matrix, checking the delay setting.

      OR

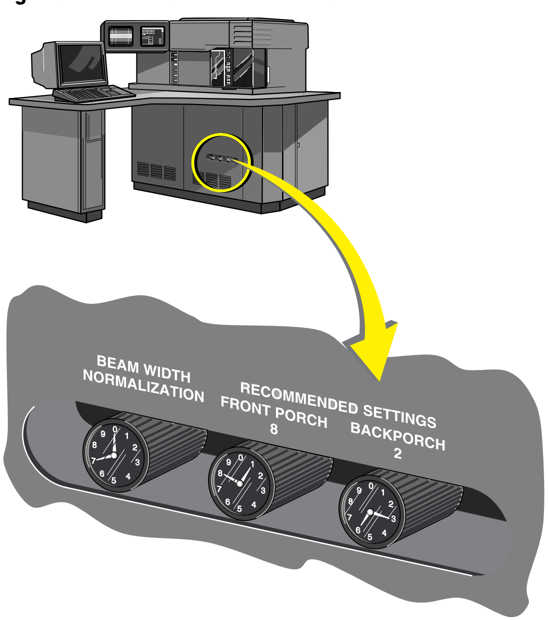
      - Run fluorospheres at the same concentration as the sample to be sorted.

10. Change the **Sort Droplet** to 3 and ensure that the side and center streams are as clean as possible by adjusting the front and back porch adjustments (Figure 4.2-2).

    Note: A change in sheath pressure may require you to readjust the porches, even if no other changes were made.

    - Check the side and center streams on the 1, 2 and 3 **Drops Sorted** settings. The streams should look good on all of these settings.
    - If the side streams do not look good, clean or change the flow cell.

Figure 4.2-2 Front and Back Porch



11. Observe the sort pulses with your scope (TP6 on Sort Oscillator card). For non-ESP systems, the front and back porch adjustments are on the Sort Oscillator card:
    a. Adjust R93 for the best side streams, while keeping the front porch level between 60 and 95% of the main pulse.
    b. Adjust R92 for the best center (waste) stream while keeping the back porch level between 5 and 30% of the main pulse.

Delay Calculation

1. Once the side streams are stable and singular, at the Cytometer Options screen, verify that the proper flow cell is selected;
    - 76 µ
    - 100 µ
    - jet-in-air.
2. Using the following initial camera setup procedure, calculate the droplet delay setting:
    a. Pan all the way to the right.
    b. Move the beam to the far right.
    c. Pan all the way to the left.
    d. If the last attached droplet is not visible, zoom to the new position and repeat steps a through c.
    e. Pan right and set the beam and flow cell edge cursors.
    f. Pan left and set Last Attached Drop, Left Drop, and Right Drop cursors.
    g. Enter the number of drops between the left and right drop.
    h. Record the total equivalent drop value, using whole numbers only.
3. After calculating the delay in the camera screen, go to the Sort screen.
4. Run the fluorospheres and set a sort gate on a fluorescence signal.
5. Verify that the droplets sorted is set to 1.
6. Set the **Sort Stop** to 100 to make it easy to see the correct drop quickly in the fluorescent microscope.
7. Run a sort seven times, once each for **Delay** settings from three less to three more than the values given on the Camera screen.
8. View the sorted drops under the fluorescent microscope.
    - The beads should be all in one drop; about 90% of the beads in one drop is fine. If so, select the drop and go to step 13.
    - If all (>90%) the beads are not in one drop, select the drop containing most of the beads. For example, for purposes of illustration, assume most of the beads are in drop 29 and some are in 30. In this case, you would select drop 29.
9. View the selected drop on the monitor and place a cursor tangent to the tip of it.

    Note: Verify that the camera view is zoomed in sufficiently to easily view the last drop and the filament connecting it to the stream.
10. To make the beads fall in the selected drop, 29 in our example, instead of 30, raise the flow cell very slightly by turning the flow cell height adjustment to raise the flow cell and stream 1/16 of a drop while watching the camera.

Note: Make sure that the fluorescence signals do not drop. In quartz, this adjustment is more sensitive than in jet-in-air.

11. Now sort the 100 beads using delays 28, 29, 30, or whatever meets your criteria. We use 28, 29, and 30 here only because of our example in step 8.
12. Check to see that most of the beads are now in 29 by looking at the drops on either side of 29:
    - If there are less than 10 beads in drop 28 or drop 30, then there is >90% in drop 29. Go to step 13.
    - If there are10 or more beads in drop 28 or drop 30, adjust the flow cell again, either up or down depending on which direction the beads went. Continue adjusting the flow cell until most or all of the beads are in the droplet of choice (>90%).

    For example, if you raised the flow cell too much, the beads will fall in droplet 28. If you did not raise it enough, some beads will fall in drop 30. If the flow cell was turned the wrong way, then most of the beads will be in drop 30. Reduce the number sorted to 20.
13. Do a sort matrix on the phase of that droplet (0.0 - 0.9 on the delay).

    Note: Several of the phases should have 20 in them.
14. Choose one phase and ensure that the side streams are good.
15. Verify the delay and phase by sorting several drops of 20 particles onto a slide and counting each.
16. For future use with this flow cell, match the camera delay with the actual delay by using the flow cell adjustment on the Camera screen.

    Note: The instrument remembers this value until the software is reloaded, at which point the value will have to be adjusted for that particular flow cell.

Monitoring the Sort

1. Zoom in on the last attached droplet so that both the droplet position and connecting neck are clearly visible.
2. Place two cursors around the last attached droplet to monitor the stream stability:
    a. Place one cursor tangent to the bottom of the last attached drop.
    b. Place the other cursor tangent to the bottom of the second to the last attached drop.
3. Occasionally view the side streams while sorting to ensure they remain clean and stable.
4. Watch the droplet in the camera while adjusting the drive to ensure that the connection between the last drop and the second to last remains in tact. The connection is optimal when the filament between two drops is very thin, yet still connected. At this point, the side streams should be at their best.
    - If the side streams appear to fan slightly, make minor adjustments to the crystal drive to improve the streams without affecting the sort.

- If the filament connecting the last attached drop breaks, decrease the **Crystal Drive**. If the filament fattens, increase the drive while viewing the side streams.
- If the side streams do not remain stable, stop the sort and investigate.

5. Press **DEBUBBLE** and/or **CLEAR** two or three times:
   - If the drop returns to the same place, the sort should be fine.
   - If the drop does not return to the same droplet, investigate further before proceeding with the sort or the purity and recovery may be affected.
6. Using the settings obtained above to sort a mixture of different brightness beads right and left (50,000 each direction).
   - For non-ESP systems, sort at 1,500 events/sec data rate with Abort ON.
   - For ESP systems, sort at 5,000 events/sec with Abort ON/Complete Abort ON.
7. Reanalyze the sorted beads to determine purity.
   - For non-ESP, purity is >95%.
   - For ESP, purity is >99%.

Sort Coincidence Verification

This procedure verifies operation of the sort coincidence circuitry. Perform this procedure as follows:

- After you replace a Sort Oscillator, Pulse Pileup Det.TOF, or Sort Delay card.
- When operation of the coincidence circuits are in question.

Verification and test of the coincidence circuitry is done by simulating the four possible coincidence events that can occur:

- Good followed by good
- Good followed by bad
- Bad followed by good
- Doublets, where the cells are too close together to be resolved as separate cells.

  Note: Doublets are a function of the PPU.

  Note: The PPU parameter (Par #1) also generates a POSITIVE PPU DETECT signal if that parameter is above 9.8 V or greater than channel 1,000.

These simulations verify the operation of the four coincidence functions:

- Sort pulse extension
- Sort pulse truncation
- Abort of two events in same droplet
- Extended abort.

To verify the operation of the four coincidence functions, you need to simulate two cells passing through the laser beam close together. The test pulse generator is used in the dual pulse mode to simulate two cells. You can vary the spacing (delay) between the pulses to simulate the spacing between the cells.

Tools/Supplies Needed:

- ❑ Oscilloscope
- ❑ DVM

Setup Pulse Generator

1. Use your oscilloscope to monitor J2 on the Pulse Generator card.
2. Go to the Cytometer Service screen (access code 26 28 32) and select **Debug 2**.
3. If **Mode** does not display **Single**, press **Mode** until it does.
4. Press the following blocks on the touchscreen:

   PORT 0 2 0 2 OUTPUT D 6 0 1 START PORT 0 2 0 4 OUTPUT F F F F START.

5. Observe the pulses on your oscilloscope:
   - If you do not observe pulses, repeat step 4.
   - If you do not observe a pair of pulses from 10 to 100 microseconds apart with a one millisecond spacing between pulse pairs, perform the Pulse Generator Card Adjustment procedure in this section.
   - If you observe pulses, go to step 6.
6. Press **PORT 0 2 0 4 OUTPUT 0 0 1 0 START**.

   Note: The observed pulse should be less than 1 V in amplitude.

   Adjust the **OUTPUT** value in the command so the pulse is between 200 mV and 1 V: (FFFF= max, 0000=min).
7. Locate and remove the signal cable coming from PMT2 going to Acquisition electronics.
8. Connect a coax cable from J2 on the Pulse Generator card to the Acquisition electronics where the PMT2 was removed.
9. At the Cytometer's Scope screen:
   a. Set **PMT2 Peak** on the lower trace.
   b. Set **PMT2 Int** on the upper trace.
   c. Set the time base to 20 microseconds/division.
10. At the Main screen, adjust **PMT 2 Gains** so the pulses are between 2 to 3 divisions high.

Acquire and Setup Sort

1. At the Workstation, select **Acquisition Parameter**.
2. Erase all parameters.
3. At the Create mode, select **PMT2 Peak** and **PMT2**.

   Note: The list of parameters in the right column should appear as PMT2 Peak, PMT2.
   - If PMT2 Peak is not the first parameter, repeat this step.
   - If PMT2 Peak is the first parameter, go to step 4.
4. At the Cytosettings screen:
   a. Set the **PMT2 Peak Discriminator** to 100.
   b. Set other channels to OFF.

5. At the Protocol screen, create two single parameter histograms to acquire PMT2 and PMT2 Peak.
6. Press F9.
7. At the Cytometer screen, put the PMT2 Peak histogram at mid scale.
8. Restart, build a histogram then **Stop**.
9. At the Workstation's Sort screen, create a sort region around the PMT2 Peak distribution and assign to Sort Right.
10. Start acquisition and send sort settings to the Cytometer.
11. At the Cytometer:
    a. Set **Sort Stop** counters to off and turn **Sort Right Enable** on and start sort right.
    b. Set **Drive Frequency** to 32 kHz.
    c. Set **Drop Sorted** to 1.
12. Verify operation of abort for two events in one drop
13. On the Cytometer Sort screen, press the **Coincidence Abort** block. This block should cycle through three states as you press it:

Coinc	Coinc	Coinc
Abort	Abort	Abort
Off	On	/PPU Det On

14. Press **Coincidence Abort** until it is in the **Coinc Abort Off** state.

    Note: Non-ESP systems will not display the PPU coincidence option. Adjust R109 on the Pulse Generator card to separate the pulses by about 80 microseconds.

    Note: At this time, the sort count should be counting and the sort rate should be about the same as the data rate.
15. Connect your oscilloscope to the alligator clip on the sample insertion rod.
16. Adjust Defection to 15% and adjust your scope to observe the sort pulses.
17. Press **Coincidence** once to switch to the **Coincidence On** state.

    Note: ESP systems can be set to either **Ext Sort** or **Comp Abort**.
18. Slowly turn R109 to reduce the time between the two pulses.

    Note: At about 30 microseconds separation between the pulse rising edges, you will see the coincidence counts increase while the sort rate decreases. This verifies operation of the abort within one drop function.

Pulse Pileup Verification

This procedure applies to ESP-equipped systems only.

1. Continue decreasing the pulse separation until the sort rate increases again. This occurs as the two pulses begin to blend and an a discriminator crossing does not occur to separate the pulses.
2. Press **Coincidence Abort** to switch to the PPU Det mode. Note that a new selection block labeled PPU sensitivity now appears. This block switches among four states:
   - 0.5 to 4 microseconds
   - 4 to 10 microseconds

- 10 to 20 microseconds
- Over 20 microseconds

Note: Choose the range that encompasses the pulse width of peak pulse being used for PPU. In this test, the 10 to 20 microsecond range should be used. In normal operation the range will depend on the flow cell:

- 76 µ jet-in-air 0.5 - 4
- 76 µ sort sense 10-20
- 100 µ sort sense 4-10

3. The sort counts stop and the abort rate increases; this verifies operation of the PPU circuit.

Verification of Sort Pulse Extension

The sort pulse is lengthened if a second good cell appears in the droplet immediately following the sort envelope for the first cell.

1. Turn **Coincidence** to **Coinc Abort OFF**.
2. Set **Drops Sorted** to 1.
3. Adjust R109 for 80 microsecond delay.
4. Observe the sort pulse on the oscilloscope. Note that the pulse consists of a front and back porch but no center level.
5. Slowly adjust R109 to decrease the pulse delay. When the pulses are approximately 60 to 30 microseconds apart, the center level for the sort pulse appear; this indicates the one drop sort is now being extended into a two drop sort.
6. Adjust R109 so the two pulses are almost touching.
7. Change **Drops Sorted** to 3.
8. Observe the 3-drop sort envelope on the oscilloscope.
9. Slowly increase the delay between the pulses with R109 while observing the sort pulse. You will see the center level of the 3 drop envelope stretch as the pulses move further apart; this verifies the sort pulse extension function.

Sort Pulse Truncation Test

This test verifies that an unwanted cell following a good cell can be aborted by shortening the sort pulse envelope. For this test the two different integral PMT2 signals will simulate the good and bad cell.

1. Adjust R53 on the Pulse Generator card so the second PMT2 Int pulses is about one division smaller than the first pulse. You are actually adjusting the second pulses width so the PMT 2 Peak pulses will remain at about the same height.
2. Adjust R109 for a 120 microsecond delay between pulses. Acquire the pulses and ensure that you get two populations on the PMT2 (int) histogram.
3. Create two new sort regions as wide as possible on the PMT2 (integral) histogram:
   a. Put region B around the left (dimmer) population.
   b. Put region C around the right (brighter) population.
4. At the Sort screen, change it to sort region B to the left and region C to the right.

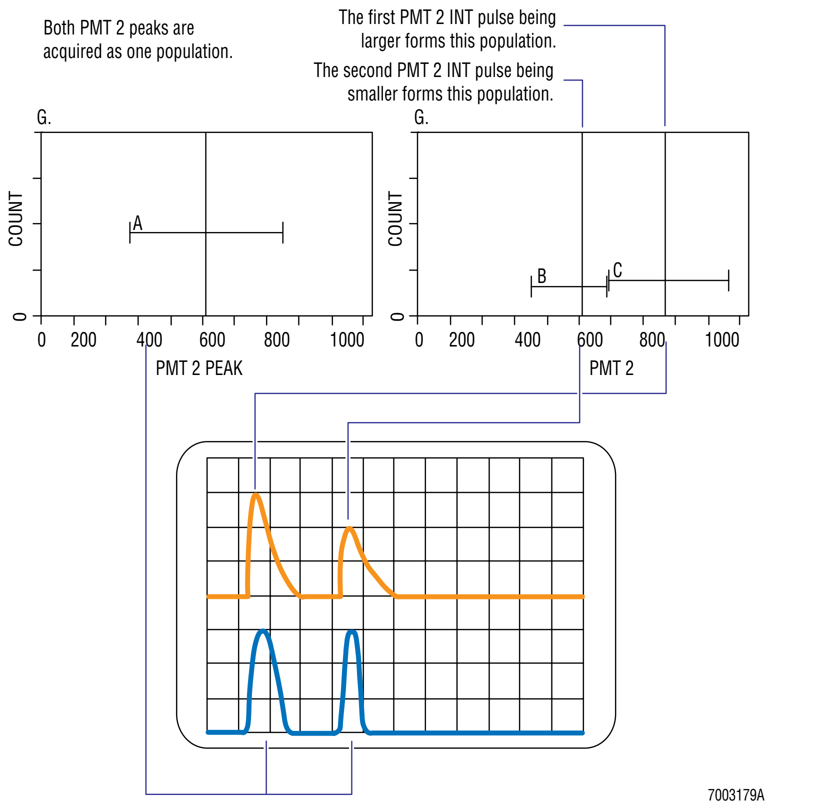
5. Send the new sort regions to the Cytometer.
6. Turn **Sort Right Enable** ON and **Sort Left Enable** OFF
7. Set **Coinc Abort** to ON and **Drops Sorted** to 3.
8. Start Sort and observe sort pulse on oscilloscope. You will see the normal 3-drop sort envelope.
9. Slowly adjust R109 to reduce the pulse delay while observing the sort pulse shape.
10. At about 90 microseconds, the three drop envelope shorten into a two drop envelope. At about 60 microseconds, the envelope shortens into a one drop envelope; this verifies operation of the truncation function.

Extended Abort Test

This procedure applies to ESP-equipped systems only and allows the system to look ahead of a good cell and abort if a bad cell is too close.

1. Adjust R109 for a 100 microsecond delay.
2. Turn **Sort Left Enable** ON and **Sort Right Enable** OFF
3. Turn **Abort** to OFF and readjust your oscilloscope to observe the sort pulses.
4. Turn **Coinc Abort** to ON. Select **Ext Sort** mode.
5. While observing the sort pulse, adjust R109 to reduce the delay until the sorting stops. Note the delay in microseconds.
6. Select **Comp Abrt** mode.
7. Slowly increase the pulse delay with R109 until sort pulses reappear. Note the delay in microseconds.
8. Verify that the delay obtained in step 7 is greater than that obtained in step 5 to verify operation of the Extended Abort mode. See Figure 4.2-3.

Figure 4.2-3 Pulse Signals and Histograms for Sort Coincidence Verification



Pulse Generator Card Adjustment

1. Put the Pulse Generator card on the extender and ensure jumpers X1, X2, X3, X4 are in place. Install jumpers if necessary.
2. Attach the scope to TP1.
3. Use the Service screen to turn on double pulses.
   a. At the Cytometer's Service screen (access code 26 28 32), select **Debug 2**.
   b. If the Mode box does not display single, press it until it does.
   c. Press the following blocks on the touchscreen: **PORT 0 2 0 2 OUTPUT D 6 0 1 START PORT 0 2 0 4 OUTPUT F F F F START**
4. Adjust the card for the values in Table 4.2-1.

Table 4.2-1 Pulse Generator Card Values

POT	Function	Test Point (TP)	Adjust For
R109	Delay	TP1	100 microsecond delay between the two pulses
R108	Rate	TP1	1 millisecond delay between pulse pairs
R54	Phase1 width	TP1	12 microsecond
R53	Phase2 width	TP1	12 microsecond (initial value)
R44	Ref volt	TP19	-10 V
R75	Gain	TP20	-5.5 V pulse
R107	Gain	TP4	Maximum undistorted pulse

The Generator is now ready to use.

Amplitude of the test pulses is determined by the value written to port 0204: FFFF will give the largest pulses and 0000 the smallest.

Pulse width is determined by the four jumpers: X1, X2, X3, X4.

- With the jumpers in, the pulses are suitable for testing the electronics in the sense-in-quartz (low bandwidth) configuration.
- With the jumpers out, the pulses are suitable for testing the sense-in-air configuration.

4.3 LINE VOLTAGE SELECTION

WARNING Risk of lethal electrical shock. Disconnect all power cords, including the instrument power cord and the air-cooled laser power cord, from the rear of the unit to prevent electrical shock.

Verify that the correct line voltage jumpers have been installed according to Table 4.3-1.

Table 4.3-1 Line Voltage Jumpers

Location	Jumpers
100 Vac input to main system transformer	TB9-2B to TB9-4B (jumper cable) TB9-7B to TB9-9B (jumper cable) TB9-1B to TB9-2B (jumper brass) TB9-6B to TB9-7B (jumper brass)
115 Vac input to main system transformer	TB9-2B to TB9-4B (jumper cable) TB9-3B to TB9-5B (jumper cable) TB9-1B to TB9-2B (jumper brass) TB9-5B to TB9-6B (jumper brass)
230 Vac input to main system transformer	TB9-1B to TB9-2B (jumper brass) TB9-3B to TB9-4B (jumper brass) TB9-5B to TB9-6B (jumper brass)
240 Vac input to main system transformer	TB9-1B to TB9-2B (jumper brass) TB9-4B to TB9-8B (jumper cable) TB9-6B to TB9-10B (jumper cable)
100 Vac input to laser auto transformer	TB10-1B to TB10-4B (jumper cable) TB10-2B to TB10-8B (jumper cable) TB10-6B to TB10-9B (jumper cable) TB10-8B to TB10-10B (jumper cable) TB10-4B to TB10-5B (jumper brass) TB10-6B to TB10-7B (jumper brass) Remove TB10-1B to TB10-10B (jumper cable)
115 Vac input to laser auto transformer	TB10-1B to TB10-10B (jumper cable)
230 Vac input to laser auto transformer	TB10-1B to TB10-4B (jumper cable) TB10-6B to TB10-9B (jumper cable) TB10-8B to TB10-10B (jumper cable) TB10-7B to TB10-8B (jumper brass) Remove TB10-1B to TB10-10B (jumper cable)
240 Vac input to laser auto transformer	TB10-1B to TB10-3B (jumper cable) TB10-6B to TB10-9B (jumper cable) TB10-8B to TB10-10B (jumper cable) TB10-7B to TB10-8B (Jumper brass) Remove TB10-1B to TB10-10B (jumper cable)

4.4 POWER SUPPLIES: MEASUREMENT AND ADJUSTMENT

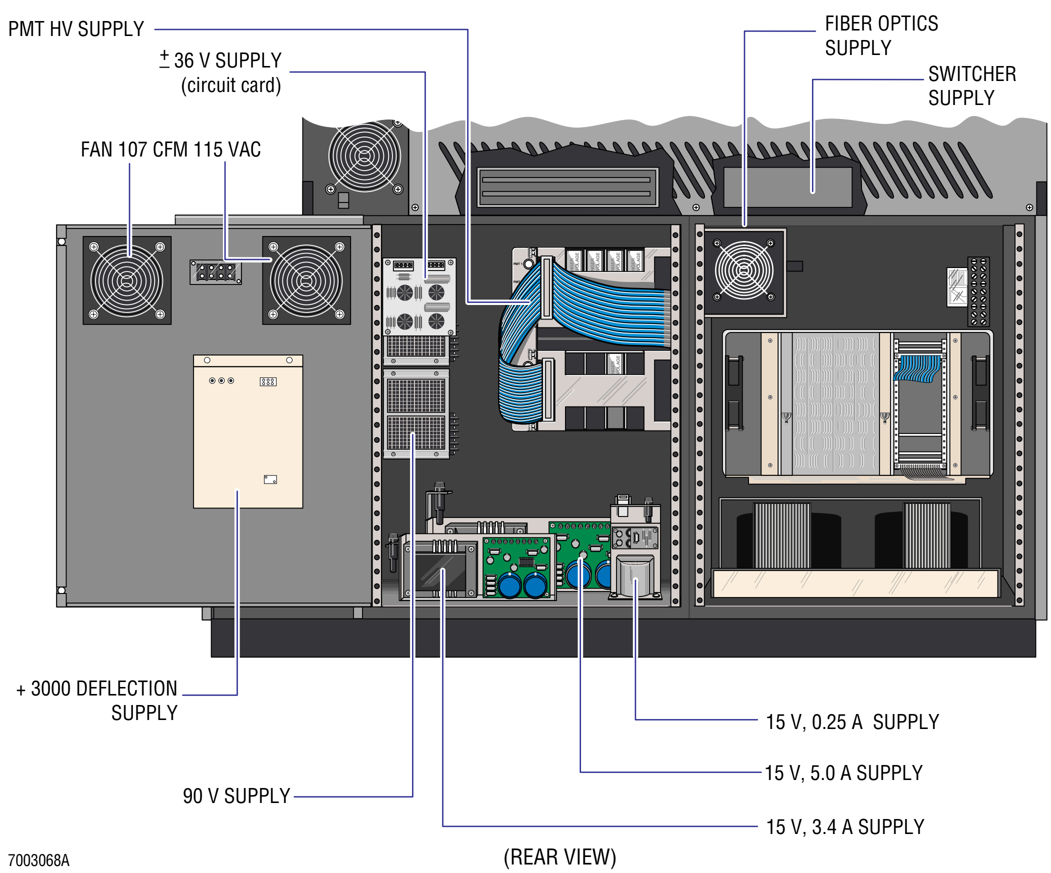
Purpose

Perform this procedure after the Pneumatic Interface card, regulator sensor, or the sheath pressure sensor is replaced. Figure 4.4-1 shows the location of the power supplies.

Tools/Supplies Needed

- ❑ Digital pressure gauge accurate to 0.01 psi
- ❑ DVM

Figure 4.4-1 Power Supplies



Regarding Figure 4.4-1:

- The PMT HV power supply is controlled by the software.
- The switcher supply provides 5 V to all logic circuits: ±12 V for multibus; and +24 V for solenoids and motors.
- The fiber optics supply should be turned to minimum intensity.
- The 90V supply goes to the Sort Output card.

- The Deflection supply is not adjustable. Test at the following test points:
  - J2 - Black = Ground
  - J3 - Blue = -2.3 to 3.0 Vdc
  - J4 - Red = +2.3 to 3.0 Vdc

Procedure

Do the measurement and adjustment procedures in Table 4.4-1.

Table 4.4-1 Power Supply Measurement and Adjustments

Power Supply	Perform
Fiber optics supply	Turn to minimum intensity
Switcher supply	Adjust to 5.25 ±0.01 Vdc between yellow and black wires
15 V, 0.25 A (PMT) supply	Adjust for +15.0 ±0.05 Vdc on orange wire; -15.0 ±0.05 Vdc on green wire. Both referenced to black wire.
15 V 5.0 A supply	Locate the Pulse Gen & Clock card in the Data Acquisition (lower) backplane far left slot, and adjust the larger ±15 Vdc Analog Power Supply using a DVM as follows: **Black DVM Lead** / **Red DVM Lead** / **DVM Result** Black test point / Orange test point / +15.0 V ±0.05 Vdc Black test point / Green test point / -15.0 V ±0.05 Vdc
15V 3.4 A supply	Adjust the ±15 Vdc 3 A power supply (connected to Bertans) with DVM connected to the HV backplane at P13 on top Bertans backplane. Put the black DVM lead on the black/white wire. Place the red DVM lead on the green wire, and adjust for -15.1 V (±0.05 Vdc). Move the red DVM lead to the orange wire. Adjust for +15.1 V ±0.05 Vdc.
90 V power supply	Adjust the power supply using the DVM as follows: **Black DVM Lead** / **Red DVM Lead** / **DVM Result** -OUT, Upper Supply / +OUT, Upper Supply / +90 Vdc ±2 Vdc -OUT, Lower Supply / +OUT, Lower Supply / +90 Vdc ±2 Vdc
Deflection power supply	Not adjustable. Test at test points: • J2- black = ground • J3 - blue = -2.3 to -3.0 Vdc • J4 - red = +2.3 to +3.0 Vdc

4.5 PNEUMATICS SYSTEM

ATTENTION: This section contains procedures for two types of systems: those with low-bleed regulators and those with non low-bleed regulators. Do not do both procedures on an instrument.

Low-Bleed Regulator Adjustment Procedure

ATTENTION: Do this procedure for low-bleed regulator instruments only.

Purpose

Do this procedure after the Pneumatic Interface card, either regulator, or the sheath pressure sensor is replaced. The following definitions appear in this procedure:

- Set pressure means enter the needed pressure on the Cytometer Control Valves screen.
- Gauge pressure refers to the pressure read on the external test pressure gauge.
- Read pressure refers to the pressure displayed on the Cytometer Control Valves screen.

Tools/Supplies Needed

- ❑ DVM
- ❑ Digital pressure gauge, 0.01 psi accuracy

Pneumatic Interface Card Adjustment

1. Access the Pneumatic Interface card:
    a. Remove the rinse bottle.
    b. Remove the rinse bottle mounting bracket.
    c. Remove the Pneumatic Interface card splash shield.
2. Connect the negative DVM test lead to the lower lead of C11 marked on the Pneumatic Interface card.
3. Place the positive DVM lead on the test points indicated in Table 4.5-1, and adjust the appropriate potentiometer for the indicated voltage.

IMPORTANT Risk of erroneous results if the voltage adjustments are made in the wrong order. Adjust the potentiometers for the indicated voltages in the order shown in Table 4.5-1.

Table 4.5-1 Potentiometer Adjustments

Adjust	For	At Test Point
R31	+10.00 ±0.01 Vdc	TP5
R34	-10.00 ±0.01 Vdc	TP1
R81	-10.00 ±0.01 Vdc	TP10
R54	+10.00 ±0.01 Vdc	TP3
R51	+8.00 ±0.01 Vdc	TP2

4. Remove the jumper from E1 to E2 and place on E2 to E3.
5. Connect the external pressure gauge to the sample pressure by plugging a T-fitting into the pinch tubing at the sample pressure pinch valve far left fitting.
6. Set Sample Pressure to 11.5 psi at the Cytometer Control Valves screen.
7. Adjust R34 slowly until the external gauge reads 11.5 ±0.02 psi. This should require no more than two turns of the potentiometer.

   If more than two turns are required, there may be a problem or your test equipment is not accurate enough for this procedure. Correct the problem and repeat steps beginning at 1.
8. Adjust R51 slowly until read pressure on Cytometer screen is 11.5 ±0.02 psi.
9. Set Sample Pressure to the following pressures and verify that the set, read and gauge pressures agree within 0.1 psi: 11.5 psi, 4.5 psi. Repeat steps 6 through 8 if needed.
10. Set Sample Pressure to 5.00 psi. Verify that the set, read and gauge pressures agree within 0.2 psi. Repeat steps 6, through 8 if needed.
11. Remove the external gauge from the sample pressure line and tee in the sheath pressure at the sheath pressure pinch valve middle fitting.
12. Set Sheath Pressure to 15.00 psi on the Cytometer screen.
13. Adjust R81 for 15.00 ±0.02 psi on the gauge.
14. Adjust R75 for 10.00 ±0.01 Vdc at TP4.
15. Set Sheath Pressure to 3.00 psi.
16. After the pressure stabilizes, change the set pressure slightly as needed for the gauge to read 3.00 psi.
17. Adjust R76 for 2.00 ±0.003 Vdc at TP4.
18. Repeat steps 11 through 14 until no further adjustment of R75 or R76 is needed.
19. Remove the jumper from E2 to E3 and place on E1 to E2.
20. Set Sheath Pressure to 12.00 psi on the Cytometer Control screen.
21. Adjust R81 for 12.0 ±0.02 psi on the gauge.
22. Set Sheath Pressure to the following pressures and verify that the set, read and gauge pressures agree within 0.1 psi: 11.5 psi, 4.5 psi. Repeat steps 13 through 20 if needed.
23. Set Sheath Pressure to 5.00 psi. Verify that the set, read and gauge pressures agree within 0.2 psi. Repeat steps 13 through 20 if needed.
24. Remove the gauge.

Low Bleed Regulator Adjustment

These regulators do not normally require adjustment. However, if difficulty is encountered with pneumatic adjustment, the regulators can be tested and adjusted as described in this procedure.

IMPORTANT Risk of erroneous results if the sample and sheath regulators are adjusted using noncalibrated test equipment. Use only calibrated test equipment to perform the following procedures.

Sample Regulator

1. Connect the DVM to TP8.
2. Connect the external gauge to the sample pressure line.
3. Set the Sample Pressure to 15.00 psi on the screen.
4. Adjust R34 for exactly 10.00 Vdc at TP8.
5. Remove the aluminum seal from regulator, and adjust the SPAN potentiometer for exactly 15.00 psi on your gauge.
6. Set the Sample Pressure to 3.00 psi.
7. Verify that the DVM reads 2.00 ±0.04 Vdc. If the DVM reads differently, replace the Pneumatic Interface card.
8. With TP8 reading 2.00 ±0.04 Vdc, adjust the ZERO potentiometer on the regulator until the gauge reads 3.00 psi.
9. Repeat steps 3 through 8 until no further adjustments are needed.
10. Disconnect the DVM from TP8.
11. Replace the seal on the regulator.

Sheath Regulator

1. Connect the DVM to TP11.
2. Remove the jumper from E1 to E2 and place on E2 to E3.
3. Connect the external gauge to the sheath pressure line.
4. Set the Sheath Pressure to 15.00 psi on Cytometer Control screen.
5. Adjust R81 for exactly 10.00 Vdc at TP11.
6. Remove the aluminum seal from regulator, and adjust the SPAN potentiometer for exactly 15.00 psi on your gauge.
7. Set the Sheath Pressure to 3.00 psi.
8. Verify that the DVM reads 2.00 ±0.04 Vdc. If the DVM reads differently, replace the Pneumatic Interface card.
9. With TP11 reading 2.00 ±0.04 Vdc, adjust the ZERO potentiometer on the regulator until the gauge reads 3.00 psi.
10. Repeat steps 4 through 9 until no further adjustments are needed.
11. Remove the DVM from TP11.
12. Replace the seal on the regulator.
13. Do Pneumatic Interface Card Adjustment.

Non Low-Bleed Regulator Adjustment Procedure

Purpose

ATTENTION: Do this procedure for non low-bleed regulator instruments only.

This procedure applies only to systems with the non-low-bleed (old type) regulators. This procedure permits minor field alignment to allow for matching the Pneumatic Interface card to the system upon card or sensor replacement. This procedure assumes the card has been previously factory adjusted.

Do this procedure after replacement of the Pneumatic Interface card, either regulator, or the sheath pressure sensor.

The following definitions appear in this procedure:

- Set pressure is the bottom pressure displayed on the Control screen, which is the pressure entered by the operator in the Service valve screen.
- Read pressure is the upper pressure displayed on the Control screen, which is the pressure sensed by the system.
- Gauge pressure is the pressure shown on the external gauge.

Tools/Supplies Needed

❑ 0-15 psi gauge

Procedure

1. If the card is not known to be system tested (factory adjusted), make the following adjustments:

Pot Number	Test Point	Voltage
R31	TP5	7.00
R34	TP1	-3.00
R51	TP2	8.00
R54	TP3	10.00

2. Display the set and read pressures at the Valves screen on the Cytometer, and verify that the sheath bottle is filled to the 2200 mL mark.
3. Connect the external 15 psi gauge to the sample pressure line with a Y-fitting at the sample pressure pinch valve at the far left fitting on the valve bracket.
4. Sequentially adjust three pots by a slight amount until the set pressure, read pressure, and gauge pressure all agree ±0.05 psi:
   a. Enter the Sample SET pressure 14 psi, and adjust R51 to match gauge with set.
   b. Adjust R34 to match read with SET.
   c. Enter sample pressure 5 psi, and adjust R59 to match read with SET.
   d. Repeat a through c as needed.

5. Connect external gauge to sheath pressure line at the sheath pressure pinch valve center fitting.
6. Sequentially adjust three pots slightly until the set pressure, read pressure, and gauge pressure all agree ±0.05 psi. Allow time for pressures to stabilize, and make a series of small adjustments to approach the final values.
   a. Set Sheath Pressure to 14.0 psi.
   b. Adjust R75 to match the read and gauge pressures. Note that both pressures change as the pot is turned; the object is to make them agree with each other not with the Sheath SET pressure.
   c. Set Sheath Pressure to 5 psi, and adjust R76 to match the read and gauge pressures.
   d. Set Sheath Pressure to 14 psi, and adjust R81 to match gauge with SET.
   e. Repeat steps b through d until no further adjustments are needed.
7. If you experience difficulty with any of these procedures, repeat the adjustments in step 1. If you continue to experience difficulty with these procedures, do Heading 4.6, ELECTRONIC PRESSURE REGULATOR ADJUSTMENT and Heading 4.7, PRESSURE SWITCH ADJUSTMENT.

4.6 ELECTRONIC PRESSURE REGULATOR ADJUSTMENT

Purpose

This procedure applies only to non low-bleed (old type) regulators.

Although electronic regulators are factory set, field adjustments may occasionally be required to set the **Range** and **Zero** adjustments on the electronic regulator as follows:

- 1 V in at the regulator produces 3 psi out
- 10 V in at the regulator produces 15 psi out.

Tools/Supplies Needed

- ❑ DVM

Procedure

1. Connect the DVM across R63 on the Pneumatic Interface card.
2. Connect external pressure gauge with a Y-connector to the sample pressure pinch valve at the far left fitting on the valve bracket.
3. At the Valves screen:
   a. Adjust Sample SET pressure until the external gauge reads 15 psi.
   b. Adjust the **Range** control until the DVM reads 10.0 V.

   Note: The maximum pressure the Pneumatic Interface card will accept is 15 psi. If the external gauge reads less than 15 psi when 15 psi is entered in this step, adjust R81 to obtain 15 psi.

   c. Adjust Sample SET pressure until the external gauge reads 3 psi.
   d. Adjust the **Zero** control until DVM reads 1.0 V.
4. Repeat step 3 until no further adjustment is needed.
5. Connect the DVM across R22 on the Pneumatic Interface card.
6. Connect the external pressure gauge with a Y-fitting to the sheath pressure line at the sheath pressure pinch valve center fitting.
7. At the Valves screen:
   a. Adjust Sheath SET pressure until the external gauge reads 15 psi.
   b. Adjust the **Range** control until the DVM reads 10.0 V.

   Note: The maximum pressure the Pneumatic Interface card accepts is 15 psi. If the external gauge reads less than 15 psi when 15 psi is entered in this step, adjust R51 to obtain 15 psi.

   c. Adjust Sample SET pressure until the external gauge reads 3 psi.
   d. Adjust the **Zero** control until DVM reads 1.0 V.
8. Repeat steps 7 until no further adjustment is needed.
9. Readjust the Pneumatic Interface card.

4.7 PRESSURE SWITCH ADJUSTMENT

Purpose

Use this procedure to adjust the system pressure switch.

Tools/Supplies Needed

- ❑ DVM

Procedure

1. Turn the unit off.
2. Connect the DVM to the two terminals on the system pressure switch while leaving the system wires attached.
3. Set the DVM for DC V.
4. Turn the unit ON.
5. Observe the DVM.

   Note: The reading is 4 to 5 V immediately after you turn the system on, then the voltage suddenly drops to 0 V after compressor pressure has built up.
6. At the Valve Screen, turn main pressure OFF.
7. Adjust the system pressure regulator until the system pressure is 30 psi.
8. Slowly reduce system pressure on the Compressor module until the DVM reads 4 to 5 V.
9. The pressure displayed on the system pressure gauge should be 17 psi. If not, adjust the screw on the system pressure switch and repeat steps 7 and 8 until the correct value is obtained.

4.8 WORKSTATION AND SOFTWARE

PC Model 486 Setup Procedure

1. At the Workstation, press Ctrl+Alt+Delete to reboot the Workstation pc.
2. During the reboot sequence, press Delete to display the Setup menu.
3. Select **STANDARD CMOS SETUP**.
4. Follow the instructions on the screen and ensure the following configuration:

```
Date (mm/date/year): current date
Time (hour/min/sec): current time
Hard Disk C:Type: 1
Hard Disk D:Type: Not Installed
Floppy Drive A: 1.2 MB 5 1/4"
Floppy Drive B:1.44 MB 3 1/2"
Primary Display: VGA/PGA/EGA
Keyboard: Installed
Base memory: 640 KB
Extended Memory: 15360 KB
Cyln       Head    WPcom   LZone   Sect    Size
306        4       128     305     17      10 MB
```

5. Press Esc to return to the Setup menu.
6. Select **ADVANCED CMOS SETUP**.
7. Follow the instructions on the screen and ensure the following configuration:

```
ADVANCE CMOS SETUP
Typematic Rate Programming: Disabled
Typematic Rate Delay (msec): 500
Typematic Rate (chars/Sec): 15
Mouse Support Option: Disabled
Above 1 MB Memory test: Enabled
Memory Test Tick Sound: Enabled
Memory Parity Error Check: Enabled
Hit <DEL> Message Display: Enabled
Hard Disk Type 47 RAM Area: 0:300
Wait For <F1> If Any Error: Enabled
System Boot Up Num Lock: On
Floppy Drive Seek At Boot: Enabled
System Boot Up Sequence: A:, C:
System Boot Up CPU Speed: High
External Cache Memory: Enabled
Internal Cache Memory: Enabled
Password Checking Option: Setup
Video ROM Shadow C000,32K: Enabled
Adapter ROM Shadow C800,32K: Disabled
Adapter ROM Shadow D000,32K: Disabled
Adapter ROM Shadow D800,32K: Disabled
Adapter ROM Shadow E000,32K: Disabled
Adapter ROM Shadow E800,32K: Disabled
System BIOS Cacheable: Enabled
Shadow RAM Write Protection: Enabled
Boot Sector Virus Protection: Disabled
```

```
External Cache Write Mode: Wr-Back
Non-Cacheable Block: AT Bus
Non-Cacheable Block Size: Disabled
Non-Cacheable Block Base: 512KB
8/16 Bit I/O Recovery Time: 4/2 CLK
Local Bus Ready Transparent: Disable
```

8. Press Esc to return to the Setup menu.
9. Select **PERIPHERAL MANAGEMENT SETUP**.
10. Follow the instructions on the screen and ensure the following:

```
On-Board Floppy Drive: Enabled
On-Board IDE Drive: Disabled
First Serial Port Address: 3f8 H
Second Serial Port Address: 2f8 H
Parallel Port Address: 378 H
```

11. Press Esc to return to the Setup menu.
12. Select **Write to CMOS and EXIT**.
13. Press Y Enter.

4.9 SOFTWARE INSTALLATION

Purpose

This procedure provides general software installation procedures. Always follow the instructions provided with the software package for specific installation instructions. This procedure affects the following serial numbers: low, ORSO9119, high, V39054.

Tools/Supplies Needed

- ❑ Software Kit, PN 6912816

Procedure

1. Install the Elite Software diskette #1 in drive B.
2. At C:\ type, `B:Install.`
3. Press Enter and follow the instructions provided.
4. Select the customer's Printer type when prompted.
5. Select **Standard** and press any key when prompted to do so until install is finished and *C:\ELITE* prompt appears.
6. Turn Workstation pc OFF and wait 10 seconds.
7. Turn Workstation pc ON and allow system to boot.
8. Install PrintQ® diskette in drive B.
9. If in Elite Software, press F2 Y Enter to exit.
10. Type `B:Install`.
11. Press Enter. *Before You Can Use PRINTQ 6 to Make Printing Fly* appears on the screen.
12. Press Enter.
13. Answer the following questions by typing the correct response and pressing . To accept the response that appears, press Enter.

...Install from Which Drive?	Type `B` and press Enter.
...Boots from Which Drive?	Type `C` and press Enter.
...Install on Which Drive?"	Type `C` and press Enter.
...Is Windows installed?	Type `NO` and press Enter.
...PrintQ Loaded each Boot?	Type `YES` and press Enter.

14. Press Enter.
15. Type the PrintQ serial number in the highlighted area. You can find the serial number on the rear cover of PrintQ User's Guide & Reference Manual.
16. Press Enter.
17. Type the requested information when prompted for:

NAME:	, and press Enter.
TITLE:	, and press Enter.
PHONE#:	, and press Enter.
COMPANY:	, and press Enter.
ADDRESS:	, and press Enter.
CITY:	, and press Enter.

18. Press Enter.
19. When PrintQ programs have been successfully installed, press Enter.
20. Select **N** for **No Auto Prompting**.
21. Select **1** for number of printers connected to computer, and press Enter.
22. Select **Printer 1 Printer Port = LPT1**, and press Enter.
23. Use to select the customer's Printer type, then press Enter.
24. To accept Printer setup, press Enter.
25. Type N for Do Not Run Test.
26. Type N for Do Not Print Registration Form.
27. Remove all diskettes from drives.
28. Press Ctrl+Alt+Delete to boot up the Elite System Software.
29. Press Enter.
30. Press F2 Enter to exit software and return to DOS.
31. Press Ctrl+Alt+P to enter the PrintQ Status Screen.
32. Press Alt+S to enter the Setup screen.
33. Type P and press Enter to select **PRINTER CONFIGURATION**.
34. Use ↓ to highlight *Interrupt* row, and press Spacebar.
35. Use ↓ to highlight *NO INTERRUPT*, and press Enter.
36. Use ↑ to highlight *Output Method* row, and press Spacebar.
37. Use ↓ to highlight *Use BIOS to OUTPUT to Printer/Plotter*, and press Enter.
38. Press F10 F10 Esc to return to the PrintQ Screen.
39. Press Alt+S to enter the Setup screen.
40. Type M to select **Manage Profiles**, and press Enter.
41. In the Print When box, select **Start printing when capture is complete**.

    Note: Do not change anything else on this screen.
42. Press Page Down to access the *Profile Selections Dialog Page screen*.
43. Use ↓ to highlight *Eject page at end* row, and press Spacebar.
44. Use ↓ to highlight *Do not eject partial last page*, and press Enter.
45. Press F10 to save changes.
46. Press Esc Esc to exit PrintQ program.
47. PrintQ is now installed. Press Ctrl+Alt+Delete to activate changes and to reboot the Workstation pc.
48. At the Protocol screen, verify *CYTOSETTINGS:SEND*.
49. Press F9. If *ALL DISCRIMINATOR SET TO OFF, CONTINUE WITH START? (Y,N)* appears, press Y Enter.

50. If the Cytometer program needs to be downloaded, *UNABLE TO RECEIVE CYTOMETER PROGRAM VERSION* appears on the screen. To download the Cytometer program:

    a. Press Enter.

    b. The question *Do You Want to Transfer the Control Files? (Y,N)* appears. Press Y Enter.

    The Cytometer Touch Screen is in increments from 0 to 400+. When the Cytometer Software is downloaded, the system resets and the Cytometer Touch Screen displays the Main Control screen.

4.10 ELECTRONIC ADJUSTMENTS: CABLE AND JUMPER CONFIGURATIONS

Purpose

This section provides the necessary procedures for you to make adjustments to the Elite's electronics.

For information on Gated Amplifier cards, refer to Heading 3.9, GATED AMPLIFIER UPGRADE.

For information on Sorting Electronics, refer to Heading 3.13, SORT PERFORMANCE (ESP) UPGRADE.

Tools/Supplies Needed

- ❑ None.

Cable Configurations

Back Panel Wiring

Verify the back panel wiring is configured as shown in the Table 4.10-1.

Table 4.10-1 Back Panel Wiring Configuration

Top AC Receptacle 16 System Power	Bottom AC Receptacle 17 Laser Power
WM71 black	WM74 black
WM72 white	WM73 white
"G" earth gnd - E13	"G" earth gnd - E14

Jumper Configurations

Multibus Card Cage

Jumper the cards in the Multibus card cage as shown in Table 4.10-2.

Table 4.10-2 Multibus Card Cage Cards, Jumper and Switch Settings

Card	Jumper and/or Switch Settings
CPU IBC 86C	Intel factory configured
Serial I/O	None
Data Taker Interface	None

Table 4.10-2 Multibus Card Cage Cards, Jumper and Switch Settings *(Continued)*

<table>
<tr><th>Card</th><th>Jumper and/or Switch Settings</th></tr>
<tr><td>256K Memory

Note: If the instrument was manufactured after 10/30/96 or if the instrument has the new computer card (PN 6706571), see the jumper settings for the External Memory card.</td><td>
<table>
<tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th></th></tr>
<tr><td>SW1</td><td>X</td><td>X</td><td>X</td><td>X</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>SW2</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td></td></tr>
<tr><td>SW3</td><td>0</td><td>0</td><td>0</td><td>X</td><td>X</td><td>0</td><td></td><td></td><td>X = Closed</td></tr>
<tr><td>SW4</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>0</td><td></td><td></td><td>0 = Open</td></tr>
<tr><td>SW5</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td></td><td></td><td></td></tr>
<tr><td>SW6</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td></td><td></td><td></td></tr>
<tr><td>SW7</td><td>X</td><td>X</td><td>X</td><td>0</td><td>0</td><td>X</td><td></td><td></td><td></td></tr>
<tr><td>SW9</td><td>0</td><td>0</td><td>0</td><td>0</td><td></td><td></td><td></td><td></td><td></td></tr>
</table>
E19 - E20 open
E25 - E26 jumpered</td></tr>
<tr><td>External Memory</td><td>X1 jumpered
X6 (a 3-pin jumper) jumpered in the +12 V position</td></tr>
<tr><td>Camera Interface</td><td>E19-E20, E21-E22, E37-E39, E38-E42, E41-E45, E44-E46</td></tr>
<tr><td>Dual CRT Control</td><td>E11-E12, E13-E14</td></tr>
<tr><td>Digiscope</td><td>E69-70,E31-39,E25-17
S1 - ALL ON.</td></tr>
<tr><td>Dual Laser Control</td><td>E4-E7, E25-E26, E28-E29, E31-E32, E34-E35, E37-E38, E40-E41</td></tr>
</table>

Non-Gated Amp Card Cage

Jumper the cards in the Non-Gated Amp card cage as shown in Table 4.10-3.

Table 4.10-3 Non-Gated Amp Card Cage, Jumper Setting

Slot	Card	Jumpers
1 (left)	Gated Amp Control - R2	E4-E5, E7-E8
7	3 PMT Sub SW-R	E9-E11
8	Dual FL SW-R	E8-E9, E11-E12
9	Scat/CV SW-R	E9-E11,E5-E7
10	Peak Scatter/Mux SW-R	E1-E2,E3-E4,E5-E7
16	Sensor Interface	E2-E3, E5-E6, E8-E9, E11-E12, E13-E14, E16-E17, E19-E20, E22-E23

Gated Amp Card Cage

Jumper the cards in the Gated Amp card cage as shown in Table 4.10-4.

Table 4.10-4 Gated Amp Card Cage, Jumper Settings

Slot	Card	Jumpers
1	Gated Amp Control R3	None
3	Dual FL SW-R	E8-E9, E11-E12
4	3 PMT Sub SW-R2	E3-E4,E9-E11
5	3 PMT Sub SW-R1	E9-E11
6	PMT Gated Amp	
7	20/40/60 Microsecond Delay 2	E1-E2, E3-E4, E10-E11 In
8	7 Microsecond Delay 2	E10-E15 In
9	7 Microsecond Delay 1	E10-E11 In
10	20/40/60 Microsecond Delay 1	E1-E2, E3-E4, E9-E10 In
11	Scat/Aux Gated Amp	
12	3 PMT Sub SW-R	E1-E2, E5-E6, E9-E11
13	Peak Scatter/Mux SW-R	E1-E2, E3-E4, E5-E7
14	Scat/CV SW-R1	E9-E11, E5-E7
16	Sensor Interface	E2-E3, E5-E6, E8-E9, E11-E12, E13-E14, E16-E17, E19-E20, and E22-E23

Data Acquisition Card Cage Configuration

Jumper the data acquisition card cage cards as shown in Table 4.10-5.

Table 4.10-5 Data Acquisition Card Cage Cards, Jumper Settings

Slot	Card	Jumpers
1(left)	Pulse Generator and Clock R	X1 through X4
5	Pulse Pileup Det./TOF	E1-E2,E20-E21
6	Mux and Scope Interface	E1-E2, E3-E4
7	Quad PSH 1	E2-E4
8	Quad PSH 2	E2-E4
9	Peak ADC PSH Control	E1-E2, E6-E7
10	Lister Out	E5-E6
11	Prism and Sort Window Test	None
12	Bitmap and Sort Decision	None
13	Interface and Scaler R	E1-E5, E2-E6, E3-E7
14	Sort Delay R3	None

Table 4.10-5 Data Acquisition Card Cage Cards, Jumper Settings

Slot	Card	Jumpers
15	Sort Oscillator R2	None
16	Sort Output R	None

Analyzer HV DAC Control Card

1. Jumper E1-E5, E2-E6, E3-E7, E4-E8.
2. Install Analyzer HV DAC Control card in the far left slot of the top Bertan card cage behind the Data Acquisition card cage.
3. Connect blue ribbon cable P85 to first (top) Analyzer HV DAC connector P1.

   Note: Pin 1 must be on top.
4. If unit has a Gated Amp/5PMT option:
   a. Ensure that the second HV DAC Control card has the following jumpers: E5-E9, E6-E10, E7-E11, E8-E12.
   b. Install the second HV DAC Control card in the bottom Bertan card cage in the far left slot.
   c. Connect blue ribbon cable P86 to the second (lower) Analyzer HV DAC connector P1.

   Note: Pin 1 must be on top.

PC Model 486

Jumper the 486 pc: first slot on the left, Lister AT card, E71-E72.

Cable Connections

Procedure

Ensure that the cables are connected as shown in Table 4.10-6 and the connectors as shown in Table 4.10-7.

Table 4.10-6 Cable Connections

From	To
Blue Ribbon Cables	
Interface and Scaler J1	P70, Pin 1 on top
Bitmap and Sort Decision J1	P38, Pin 1 on bottom
Lister Out J1	P43, Pin 1 on bottom
Mux and Scope J27	P88, Pin 1 on top
Sensor Interface J9	P76, Pin 1 on top
Gated Amp Control J1	P69, Pin 1 on top
CPU J1	P80, Pin 1 to right

Table 4.10-6 Cable Connections *(Continued)*

From	To
CPU Card J2	P92, Pin 1 to left
Serial I/O J1	P74, Pin 1 to left
Serial I/O J2	P93, Pin 1 to left
Digiscope J1A	P87, Pin 1 to right
Data Taker Interface J1	P37, Pin 1 to right
Data Taker Interface J2	P84, Pin 1 to right
Sort Bracket J1	P2 Sort Osc-R2 Card
Other Cables	
Dual CRT Power Cable P65	Position P65 on Dual CRT Pivot Mount.
Mux and Scope Card Cable Connections	
Mux and Scope J1	Pulse Pileup Det./TOF J1
Mux and Scope J2	Quad PSH 1 J3
Mux and Scope J3	Quad PSH 1 J5
Mux and Scope J4	Quad PSH 1 J7
Mux and Scope J5	Quad PSH 2 J1
Mux and Scope J6	Quad PSH 2 J3
Mux and Scope J7	Quad PSH 2 J5
Mux and Scope J8	Pulse Pileup Det./TOF J3
Mux and Scope J9	Digiscope LT
Mux and Scope J30	Digiscope UT
Quad PSH 1 Card	
Quad PSH 1 J1	Pulse Pileup Det./TOF J2
Quad PSH 1 J2	ADC J1
Quad PSH 1 J4	ADC J2
Quad PSH 1 J6	ADC J3
Quad PSH 1 J8	ADC J4
All Units	
Quad PSH 2 J7	Pulse Pileup Det./TOF J4
Quad PSH 2 J8	Pulse Pileup Det./TOF J5

Table 4.10-6 Cable Connections *(Continued)*

From	To
Quad PSH 2 J2	ADC J5
Quad PSH 2 J4	ADC J6
Quad PSH 2 J6	ADC J7
Pulse Pileup Det./TOF J6	ADC J8
ADC (7 in. cable) J9	ADC J17
Sensor Interface J1	Pneu J4
Sensor Interface J2	Pneu J3
Sensor Interface J3	Multibus CPU J1
Sensor Interface J4	Multibus CPU J2
Non-Gated Amp Units	
Scat/CV J10	Peak Scatter/Mux J12
Peak Scatter/Mux J14	Mux and Scope J9
Scat/CV J4	Mux and Scope J10
Scat/CV J3	Mux and Scope J11
Scat/CV J2	Mux and ScopE J12
Dual FL J3	Mux and Scope J13
Dual FL J4	Mux and Scope J14
Dual FL J2	Mux and Scope J15
Dual FL J7	Mux and Scope J16
Dual FL J8	Mux and Scope J17
Dual FL J6	Mux and Scope J18
3 PMT Sub J8	Mux and Scope J19
3 PMT Sub J7	Mux and Scope J20
3 PMT Sub J6	Mux and Scope J21
3 PMT Sub J4	Dual FL J1
3 PMT Sub J5	Dual FL J5
Gated Amp Units	
7 Microsecond Delay 2 (slot 8) J2	20/40/60 Microsecond Delay 2 (slot 7) J2
7 Microsecond Delay 2 (slot 8) J4	20/40/60 Microsecond Delay 2 (slot 7) J5
7 Microsecond Delay 2 (slot 8) J6	20/40/60 Microsecond Delay 2 (slot 7) J8

Table 4.10-6 Cable Connections *(Continued)*

From	To
7 Microsecond Delay 2 (slot 8) J8	20/40/60 Microsecond Delay 2 (slot 7) J11
20/40/60 Microsecond Delay 2 (slot 7) J1	PMT Gated Amp (slot 6) J8
20/40/60 Microsecond Delay 2 (slot 7) J4	PMT Gated Amp (slot 6) J9
20/40/60 Microsecond Delay 2 (slot 7) J7	PMT Gated Amp (slot 6) J10
20/40/60 Microsecond Delay 2 (slot 7) J10	PMT Gated Amp (slot 6) J11
20/40/60 Microsecond Delay 2 (slot 7) J3	PMT Gated Amp (slot 6) J13
20/40/60 Microsecond Delay 2 (slot 7) J6	PMT Gated Amp (slot 6) J14
20/40/60 Microsecond Delay 2 (slot 7) J9	PMT Gated Amp (slot 6) J17
20/40/60 Microsecond Delay 2 (slot 7) J12	PMT Gated Amp (slot 6) J18
20/40/60 Microsecond Delay 2 (slot 8) J1	PMT Gated Amp (slot 6) J12
7 Microsecond Delay 2 (slot 8) J3	PMT Gated Amp (slot 6) J15
7 Microsecond Delay 2 (slot 8) J5	PMT Gated Amp (slot 6) J16
7 Microsecond Delay 2 (slot 8) J7	PMT Gated Amp (slot 6) J19
Bundle these cords and continue.	
7 Microsecond Delay 1 (slot 9) J8	20/40/60 Microsecond Delay 1(slot 10) J11
7 Microsecond Delay 1 (slot 9) J6	20/40/60 Microsecond Delay 1(slot 10) J8
7 Microsecond Delay 1 (slot 9) J4	20/40/60 Microsecond Delay 1(slot 10) J5
7 Microsecond Delay 1 (slot 9) J2	20/40/60 Microsecond Delay 1(slot 10) J2
20/40/60 Microsecond Delay 1(slot 10) J1	Scat/Aux Gated Amp (slot 11) J6
20/40/60 Microsecond Delay 1(slot 10) J4	Scat/Aux Gated Amp (slot 11) J7
20/40/60 Microsecond Delay 1(slot 10) J7	Scat/Aux Gated Amp (slot 11) J8
20/40/60 Microsecond Delay 1(slot 10) J10	Scat/Aux Gated Amp (slot 11) J9
20/40/60 Microsecond Delay 1(slot 10) J3	Scat/Aux Gated Amp (slot 11) J11
20/40/60 Microsecond Delay 1(slot 10) J6	Scat/Aux Gated Amp (slot 11) J12
20/40/60 Microsecond Delay 1(slot 10) J9	Scat/Aux Gated Amp (slot 11) J15
20/40/60 Microsecond Delay 1(slot 10) J12	Scat/Aux Gated Amp (slot 11) J16
7 Microsecond Delay 1 (slot 9) J1	Scat/Aux Gated Amp (slot 11) J10
7 Microsecond Delay 1 (slot 9) J3	Scat/Aux Gated Amp (slot 11) J13
7 Microsecond Delay 1 (slot 9) J5	Scat/Aux Gated Amp (slot 11) J14
7 Microsecond Delay 1 (slot 9) J7	Scat/Aux Gated Amp (slot 11) J17

Table 4.10-6 Cable Connections *(Continued)*

From	To
PMT Gated Amp (slot 6) J20	3 PMT Sub SW-R1 (slot 5) J3
PMT Gated Amp (slot 6) J21	3 PMT Sub SW-R1 (slot 5) J2
PMT Gated Amp (slot 6) J22	3 PMT Sub SW-R1 (slot 5) J1
PMT Gated Amp (slot 6) J23	3 PMT Sub SW-R2 (slot 4) J1
PMT Gated Amp (slot 6) J24	3 PMT Sub SW-R2 (slot 4) J2
PMT Gated Amp (slot 6)J25	3 PMT Sub SW-R2 (slot 4) J3
3 PMT Sub SW-R1 (slot 5) J5	Dual FL (slot 3) J1
3 PMT Sub SW-R2 (slot 4) J5	Dual FL (slot 3) J5
Scat/Aux Gated Amp (slot 11) J18	Peak Scatter/Mux (slot 13) J11
Scat/Aux Gated Amp (slot 11) J19	3 PMT Sub SW-R3 (slot 12) J1
Scat/Aux Gated Amp (slot 11) J20	3 PMT Sub SW-R3 (slot 12) J2
Scat/Aux Gated Amp (slot 11) J21	3 PMT Sub SW-R3 (slot 12) J3
3 PMT Sub SW-R3 (slot 12) J5	Peak Scatter/Mux (slot 13) J1
3 PMT Sub SW-R3 (slot 12) J6	Peak Scatter/Mux (slot 13) J8
3 PMT Sub SW-R3 (slot 12) J7	Peak Scatter/Mux (slot 13) J6
3 PMT Sub SW-R3 (slot 12) J8	Peak Scatter/Mux (slot 13) J5
3 PMT Sub SW-R3 (slot 12) J4	Scat/CV SW-R1 (slot 14) J1
Peak Scatter/Mux (27 in. cable) (slot 13) J2	Scat/CV (slot 14) J6
Peak Scatter/Mux (27 in. cable) (slot 13) J3	Scat/CV (slot 14) J5
Peak Scatter/Mux (27 in. cable) (slot 13) J9	3 PMT Sub SW-R1 (slot 5) J6
PMT Gated Amp (27 in. cable) (slot 6) J2	Scat/Aux Gated Amp (slot 11) J4
PMT Gated Amp (27 in. cable) (slot 6) J3	Scat/Aux Gated Amp (slot 11) J5
Scat/CV (27 in. cable) (slot 14) J8	Dual FL (slot 3) J6
Scat/CV (27 in. cable) (slot 14) J10	Peak Scatter/Mux (slot 13) J12
Gated Amp Control R3 (27 in. cable) (slot 1) J3	Scat/Aux Gated Amp (slot 11) J1
Gated Amp Control R3 (27 in. cable) (slot 1) J4	PMT Gated Amp (slot 6) J1
Mux and Scope J29	Digiscope LT
Mux and Scope J30	Digiscope UT
Mux and Scope J26	No Connection
Mux and Scope (27 in. cable) J9	Peak Scatter/Mux (slot 13) J14

Table 4.10-6 Cable Connections *(Continued)*

From	To
Mux and Scope (27 in. cable) J10	Scat/CV (slot 14) J4
Mux and Scope (27 in. cable) J11	Scat/CV (slot 14) J3
Mux and Scope (27 in. cable) J12	Scat/CV (slot 14) J2
Mux and Scope (27 in. cable) J13	3 PMT Sub SW-R1(slot 5) J8
Mux and Scope (27 in. cable) J14	3 PMT Sub SW-R1(slot 5) J7
Mux and Scope (27 in. cable) J15	Peak Scatter/Mux (slot 13) J4
Mux and Scope (27 in. cable) J16	Dual FL (slot 3) J3
Mux and Scope (27 in. cable) J17	Dual FL (slot 3) J4
Mux and Scope (27 in. cable) J18	Dual FL (slot 3) J2
Mux and Scope (27 in. cable) J19	Dual FL (slot 3) J7
Mux and Scope (27 in. cable) J20	Dual FL (slot 3) J8
Mux and Scope (27 in. cable) J21	Scat/CV (slot 14) J9
Mux and Scope (27 in. cable) J22	3 PMT Sub SW-R2 (slot 4) J8
Mux and Scope (27 in. cable) J23	3 PMT Sub SW-R2 (slot 4) J7
Mux and Scope (27 in. cable) J24	Peak Scatter/Mux (slot 13) J10
Mux and Scope (27 in. cable) J25	Gated Amp Control R3 (slot 1) J2
Mux and Scope (27 in. cable) J31	Gated Amp Control R3 (slot 1) J6
Gated Amp Units: Interconnections from Sensor Area	
FALS (WM400)	Scat/Aux Gated Amp (slot 11) J2
PMT1	ac Filter Coax Assembly (PN 6586479-7)
ac Filter Coax Assembly (PN 6586479-7)	Scat/Aux Gated Amp (slot 11) J3
PMT2	PMT Gated Amp (slot 6) J4
PMT3	PMT Gated Amp (slot 6) J5
PMT4	PMT Gated Amp (slot 6) J6
PMT5	PMT Gated Amp (slot 6) J7 (If applicable)
Non-Gated Amp Units: Interconnections from Sensor Area	
FALS (WM400)	Peak /Scatter Mux SW-R (slot 10) J11
PMT1	ac Filter Coax Assembly (PN 6586479-7)
ac Filter Coax Assembly (PN 6586479-7)	Scat/CV (slot 9) J1
PMT2	3 PMT Sub (slot 7) J1

Table 4.10-6 Cable Connections *(Continued)*

From	To
PMT3	3 PMT Sub (slot 7) J2
PMT4	3 PMT Sub (slot 7) J3

Table 4.10-7 Cable Connectors

From	To
P99	Sort Transistor Bkt J2
P59	Dual Crt Controller J2
P61	Dual Crt Controller J3
J43	Dual Crt Controller J9
P60	Digiscope J2
P62	Digiscope J3
J44	Camera Interface J5
P95	Camera Interface J6
Camera Interface Cable	Camera Interface J1
P49	Dual Laser Controller J3
P89	Dual Laser Controller J4

4.11 SORT SENSE FLOW CELL ADJUSTMENT AND VERIFICATION

Purpose

Use this procedure to adjust the sort sense flow cell on the following instruments:

- Elite, 2362
- Elite ESP, 2363

Refer to the Special Procedures and Troubleshooting manual for additional information on flow cell adjustments.

Tools/Supplies Needed

- ❑ Elite ESP sort sense flow cell, 76 µ, PN 6859313-1
- ❑ Elite ESP sort sense flow cell, 100 µ, PN 6859300-0
- ❑ Sort sense flow cell, 76 µ, PN 6856511-1
- ❑ Sort sense flow cell, 100 µ, PN 6856101-9
- ❑ Barbed fittings (5), 0.115 i.d. to 0.180 PVC, PN 6232418-0
- ❑ Barbed fittings (5) (6 for instruments with Autoclone Sorting Option, 0.062 i.d. to 0.062 PVC, PN 6232417-1
- ❑ Sheath filter, 0.2 m, with fittings, PN 6858525-2
- ❑ Flow cell body with fixed insertion rod, PN 6858366-7
- ❑ DVM
- ❑ 50% bleach solution, 2 L
- ❑ Fluorospheres
- ❑ IsoFlow sheath fluid, PN 8547008-6
- ❑ Deionized water
- ❑ Microscope
  - Fluorescent microscope is recommended
  - Light microscope if fluorescent microscope is not available

Procedure

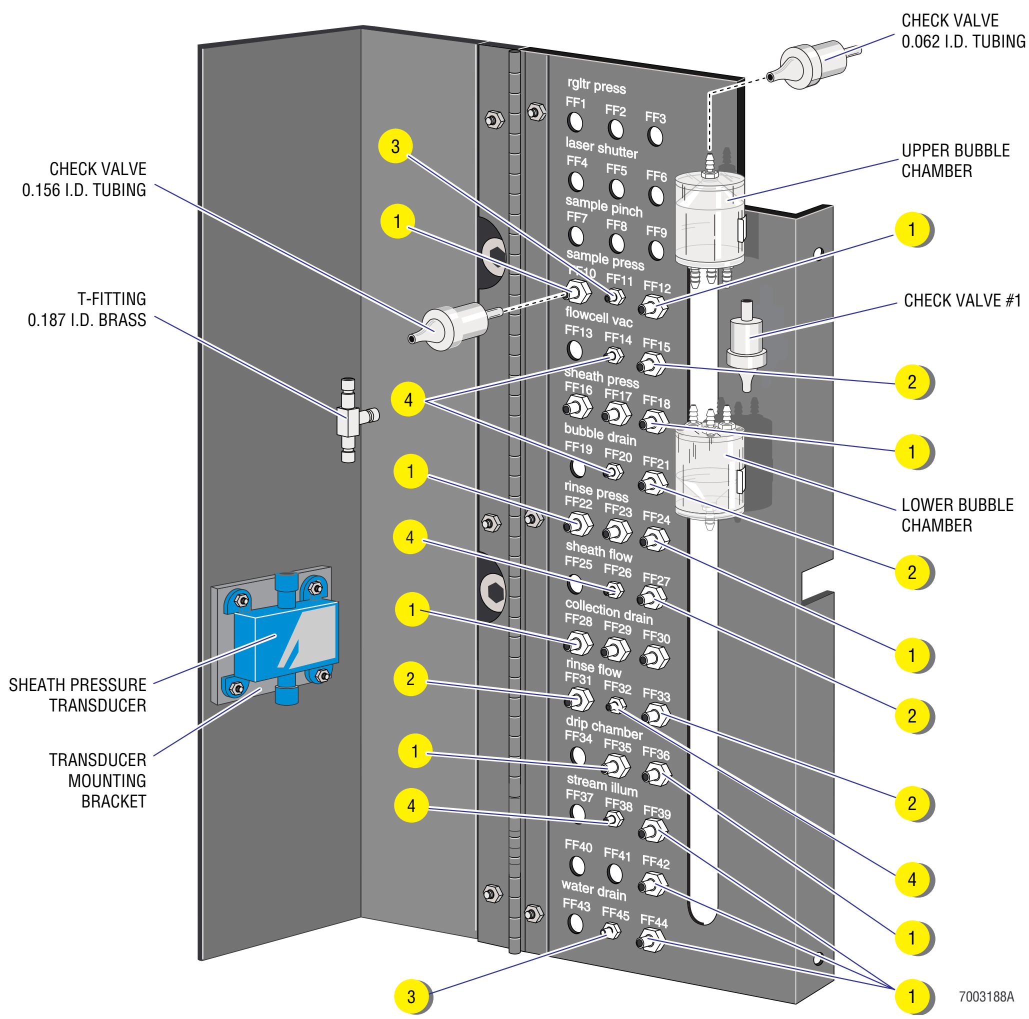
ATTENTION: The instrument must be in proper working order, with no sheath, sample, system, or regulator pressure leaks.

Before changing the flow cell, verify there are no metal barbed fittings on the valve bracket assembly. See Figure 4.11-1. If there are metal fittings on this assembly, or if there is a blackish/greenish buildup where the insertion rod enters the flow cell body, do Replacing Metal Fittings with PVC Fittings before replacing and testing the new flow cell.

Replacing Metal Fittings with PVC Fittings

1. Verify that fittings marked 2 and 4 in Figure 4.11-1 are PVC, not metal, fittings.
   a. If the fittings are metal, replace with PVC fittings.
   b. If the fittings are PVC but the flow cell body exhibits a cloudy blackish/greenish substance near the point where the insertion rod enter the flow cell body, there may be a defective PVC fitting causing a voltage leak during sorting and electrolyzing the insertion rod. Go to step 2.
   c. If the fittings are PVC and there is no cloudy blackish/greenish substance, go to step 3.

Figure 4.11-1 Fittings on Valve Assembly Bracket

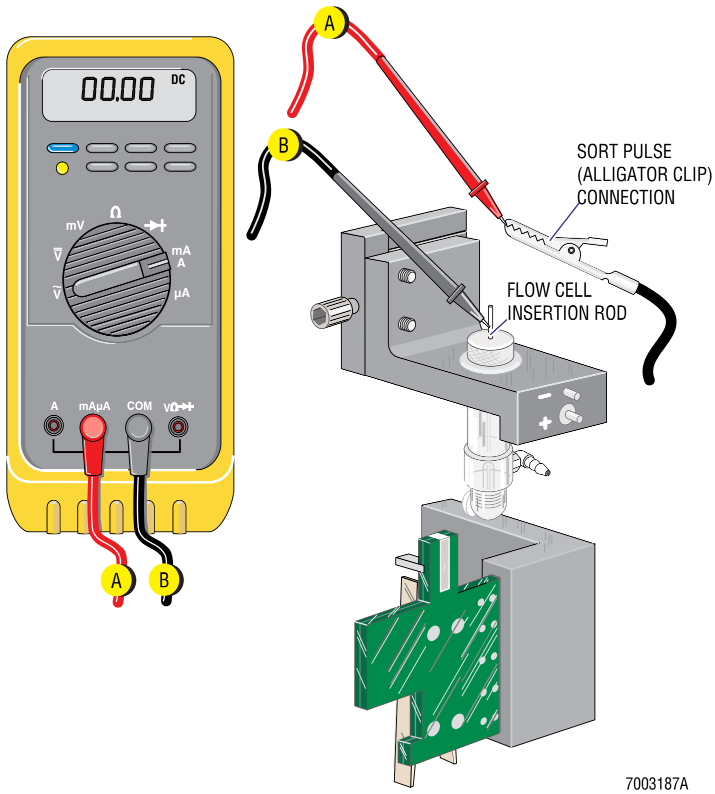


Regarding Figure 4.11-1:

- Item 1 is a large metal barbed fitting, 0.115 i.d. to 0.180 id.
- Item 2 is a large PVC barbed fitting, 0.115 i.d. to 0.180 i.d.
- Item 3 is a small metal barbed fitting, 0.062 i.d.
- Item 4 is a small PVC barbed fitting, 0.062 i.d.

2. Locate and replace the defective PVC fitting:
   a. Press **SHEATH**.
   b. Verify **Sort Pulse Enabled** is off.
   c. Verify **Deflection** is 0.00%.
   d. Disconnect the clip from the flow cell introduction rod and connect a multimeter in series to measure the current. See Figure 4.11-2.

Figure 4.11-2 Connecting Clip from Flow Cell to DVM



   e. Turn the stream deflection knob, located on the sample station next to the mixer speed control, all the way right or left.
   f. Locate the incorrect, cracked, or leaking fitting:
      1) Determine if there is current by increasing **Deflection** and checking your meter for any current indication.
      2) If current is detected, troubleshoot by pinching the tubing carrying the fluid to and from the flow cell until the current reading drops.
   g. Replace the defective fitting.
3. Decontaminate the system:
   a. Remove the 0.2 m sheath filter and bypass it with a piece of tubing equipped with the correct fittings.
   b. Fill the sheath tank with the 50% bleach solution.

c. Replace the flow cell with the biohazard flow cell included in the customer's accessories box.

d. Allow the system to run in sheath until the sheath tank is about one-quarter full.

   Note: This should take about 45 minutes.

e. Rinse the sheath tank thoroughly and fill with deionized water.

f. Allow the system to run in sheath again for the same amount of time as in step d.

g. Fill the sheath tank with Isoflow and leave system in sheath for 5 minutes to get the Isoflow sheath fluid throughout the system.

h. Put the system in vacuum.

i. Do Flow Cell Replacement and Verification.

j. Remove the biohazard flow cell and the flow body.

k. Install the new flow body and the new sort sense flow cell that the customer will be using.

l. Install the new 0.2 m sheath filter and ensure that it fills immediately with sheath.

m. Release any air bubbles in the filter using the sheath purge valve.

Flow Cell Replacement and Verification

Consider a flow cell defective and fail it only when:

- There is no discernible fluidics problem in the system.
- Stable breakoff cannot be achieved. The droplets appear to constantly drift up and down stream.
- Seven or eight singular, good side streams cannot be attained out of ten.
- The flow cell has been tightened sufficiently
- **CLEAR** and/or **DEBUBBLE** does not alleviate the instability in the droplets.
- There are no bubbles in the flow cell.
- The correct fittings in the pneumatics manifold are PVC and there is no electrolysis occurring at the point that the insertion rod enters the flow body. Electrolysis is evidenced by a buildup of a blackish/greenish cloudy substance.

1. Verify that there are no metal barbed fittings on the valve bracket assembly and that there is not a blackish/greenish buildup where the insertion rod enters the flow cell body.

   a. If either of these conditions exist, do Replacing Metal Fittings with PVC Fittings before replacing and testing the new flow cell.

   b. If neither of these conditions exist, go to step 2.

2. Power up the system, including the laser and valves.

3. Verify that the system is at 12.0 psi.

4. Verify that the system is in vacuum.

5. Remove the new flow cell from the vial and unwrap the tissue.

   Note: The Certification Form included with the flow cell contains the crystal drive and frequency settings that gave the most stable droplet breakoff with clean crisp side

streams and at least one free drop above the ground plate. (In house test unit results may vary slightly.)

6. Inspect the flow cell under the microscope and verify that the orifice is clean and round, and in the center of the square channel that is visible behind it.
   - If the flow cell appears dirty or cloudy, clean with canned air or rinse with distilled water and dry completely.
   - If the flow cell appears clean and clear, go to step 7.

IMPORTANT Risk of compromising fluorescence sensitivity. If you touch the lens or quartz portion of the flow cell with your finger or anything else, fluorescence sensitivity may be compromised. Do not touch lens or quartz of flow cell.

7. Screw on the new flow cell and finger tighten onto the flow body. Be careful not to touch the lens or quartz portion of the flow cell.

IMPORTANT Risk of inconsistent sheath flow and inconsistent stability. If the tubing coming out of the flow cell body is bound or kinked, sheath flow and stability inconsistencies may occur. Ensure the tubing is not bound or kinked.

8. Check the sheath and flush tubing on the two ports on the flow cell body and verify the tubing:
   a. Does not bind or kink.
   b. Is free and clear of the cables and the bezel.
9. Remove the beam shaper and align the flow cell so the reflection back to the laser interlock is just slightly off to one side.
10. Replace the beam shaper.
11. Run fluorospheres and align the system. Use the flow cell and beam shaper adjustment knobs to ensure that the new flow cell is optimally aligned with the maximum fluorescence and forward scatter intensity possible, and that half peak CVs are as low as possible (at least under 2.0%).
12. After acceptable alignment, press **SHEATH** to stop the beads from running out.
13. Enter standard values for the sort set up functions:
   a. Set **Drive Frequency** to 28-32 kHz.
   b. Set **Crystal Drive** to 65-75%.
   c. Set **Deflection** amplitude to 75-85%
14. Place the ground plate between the bottom of the beam shaper lens and the bottom of the beam shaper itself.

    Note: You can vary this a bit as long as it is not too high to cut off the laser signal into the flow cell.
15. Set up the camera to view the droplets:
   a. Zoom in enough to see that the drops are crisp and in focus.
   b. Look above and below the ground plate.

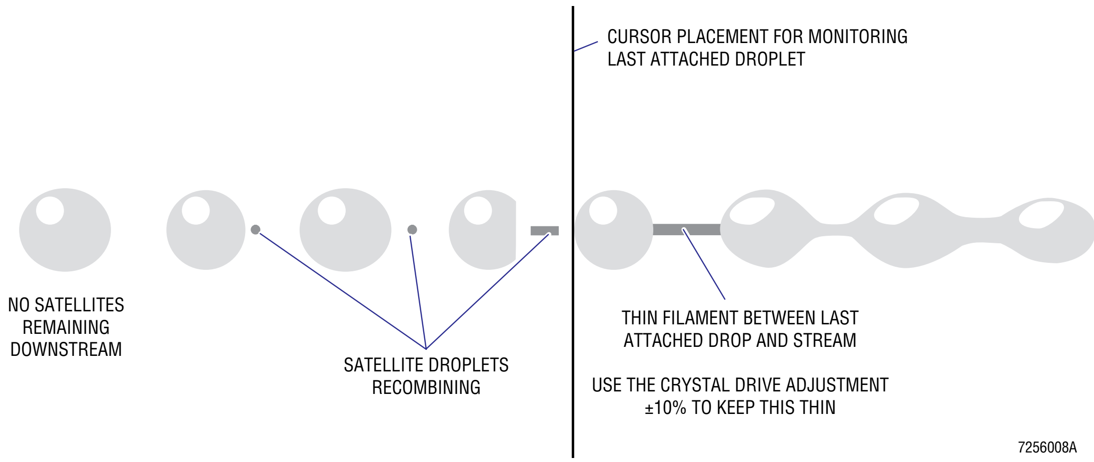
16. Scroll through the **Drive Frequency** to find the best possible frequency that produces crisp, clean droplets and a high enough droplet breakoff so that it is slightly above the ground plate.

    If the drops look crisp and focused, but the last attached drop is not above the ground plate, either move the plates slightly to see the last droplet, or increase the **Crystal Drive** slightly to achieve the same thing.

    Note: Avoid increasing the **Crystal Drive** above 90%. It is not necessary to have three free drops above the ground plate. One drop is sufficient and will not affect your sort in any way. As long as the breakoff between the last attached drop and the first free drop is visible, the sort will be easy to monitor.

17. Ensure that the filament which connects the last attached drop to the one above it is thin; it should be thin enough to be connected, but not broken off. See Figure 4.11-3.

Figure 4.11-3 Filament Connecting Last Attached Drop



18. When the drops look sharp, crisp and in focus all the way down stream, and there is sufficient space above the ground plate (at least one droplet):
    a. Turn on the **Sort Left**.
    b. Turn on **Sort Right**.
    c. Activate the sort test.
    d. Turn on the **High Voltage**.

19. Ensure that 3-drops sorted is selected and view the side streams.

    If the light is not sufficient to see the streams adequately, adjust the light so the side streams can be monitored at a glance.

20. Using the phase adjustment of the droplet delay button, scroll through the settings and ensure that at least seven or eight out of the 10 settings produce tight, clean, singular side streams.
    a. If the streams are acceptable, go to step 21.
    b. If the streams are not acceptable:
        1) Check the filament between the last attached and the next one up. If the filament is not acceptable, adjust crystal drive.

2) Check the flow cell and/or flow cell body. Turn off the HV to the sort plates and press **CLEAR** two times. After the droplets return to their position, check the side streams again. If they do not return to the same position, there may have been a clog or air bubble in the flow cell.

3) Verify the crystal frequency setting to ensure that it was not set incorrectly due to a fluidics problem.

4) Correct the fluidics problem, if applicable. Stability is very important. If the last droplet still does not return to the position that it was before a **CLEAR** or **DEBUBBLE** was done, there may be a fluidics problem.

21. Set the cursors to calculate the delay:

    a. Zoom the camera out to get the full pan function to reach the laser intersection point and the first free droplet.

    b. Place the cursors. Refer to the Operator's manual for instructions.

22. Verify the flow factor adjustment by performing a matrix and determining the correct delay at the Sort screen.

23. Do Delay Calculation under Heading 4.2, SORT WAVEFORM VERIFICATION AND ADJUSTMENT PROCEDURE.

24. If you were doing Replacing Metal Fittings with PVC Fittings, resume that procedure at step j.

4.12 OPTICAL ALIGNMENT PROCEDURE

Purpose

Use this procedure to align the flow cell and the laser beam. The procedure is divided into three sections: Flow Cell Alignment, Laser Beam Alignment, and Optical Fine Tuning.

Tools/Supplies Needed

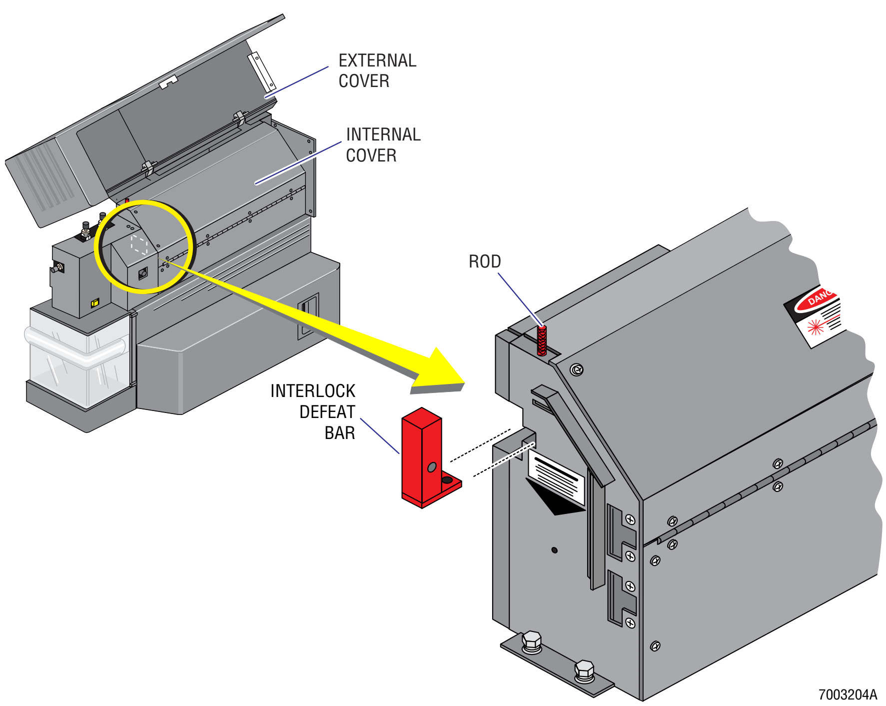
- ❑ None.

Flow Cell Alignment

WARNING Risk of personal injury. The laser beam can cause eye damage if viewed either directly or indirectly from reflective surfaces (such as a mirror or shiny metal surface). To prevent eye damage, avoid direct exposure to the beam. Do not view it directly or with optical instruments except with special service tools as directed in this manual.

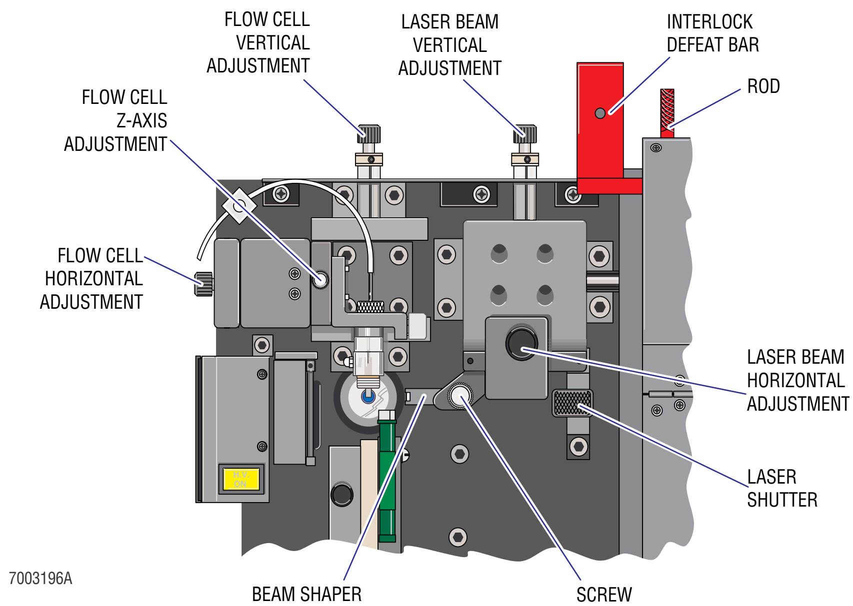
1. Press **VACUUM**.
2. Insert the interlock defeat bar (Figure 4.12-1):
   a. Pull rod up.
   b. Open the external cover.
   c. Insert the red interlock.
   d. Push rod down to secure interlock defeat bar.

Figure 4.12-1 Interlock Defeat Bar



3. Remove the lens block. See Figure 4.12-2 for location:
   a. Loosen the screw.
   b. Remove the beam shaper.

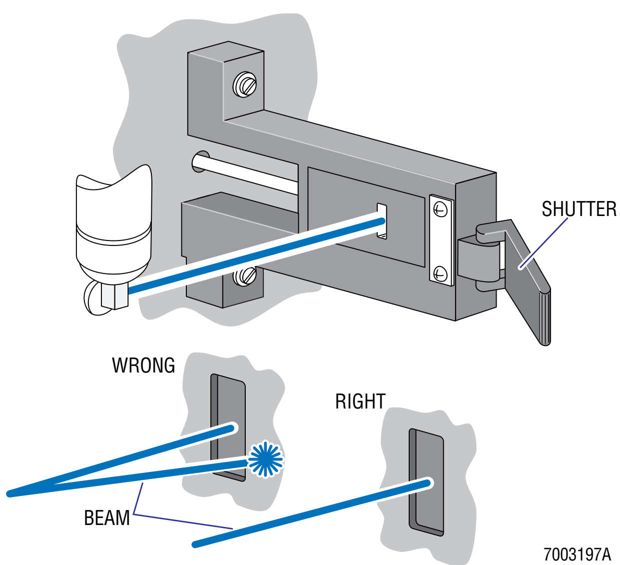
Figure 4.12-2 Location of Optical Alignment Components



4. Open the laser shutter.

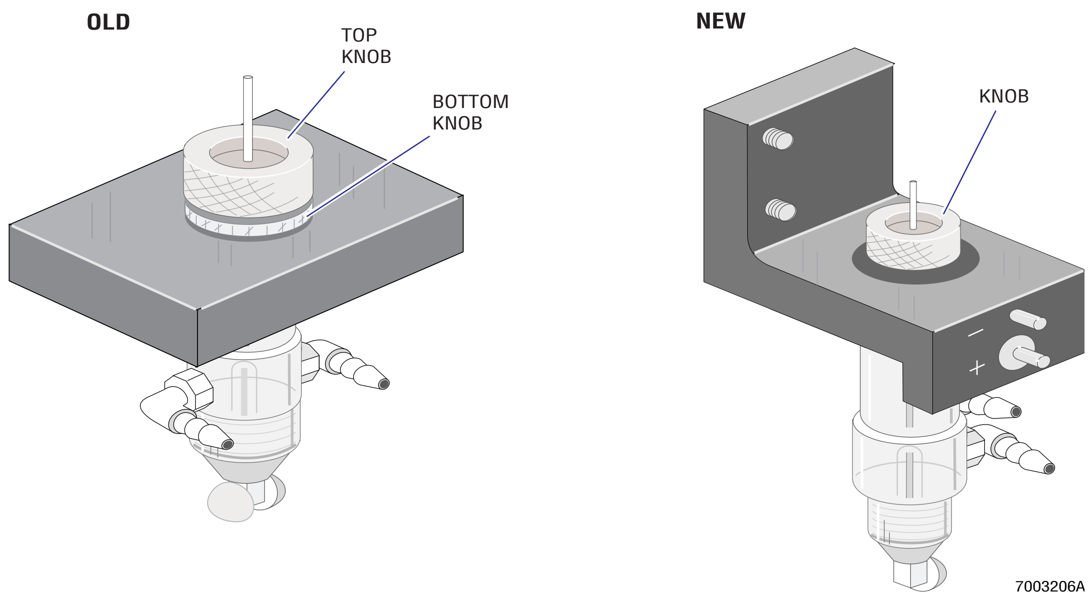
   Note: The flow cell is now rotated so the laser beam strikes perpendicular to the right side of the tip. A correctly rotated flow cell directs a small amount of reflected light back through or slightly off the center of the shutter hole as shown in Figure 4.12-3.

Figure 4.12-3 Laser Beam Reflected through Shutter Hole



5. Rotate the flow cell chamber on the Elite:
    a. For an old flow cell body (Figure 4.12-4):
        1) Loosen the top knob.
        2) Remove the knob and insertion rod.
        3) Loosen the bottom knob slightly.
        4) Rotate the flow chamber until the beam reflects back onto itself.
        5) Tighten the knob.
        6) Hold the flow cell in place and replace the insertion rod.
        7) Tighten the top knob.

Figure 4.12-4 Flow Cell Bodies, Old and Newv



    b. For a new flow cell body (Figure 4.12-4):
        1) Loosen the knob slightly.
        2) Rotate the flow cell chamber until the laser beam reflects back onto itself.
        3) Tighten the knob.
        4) Replace the beam shaper and tighten the screw.

ATTENTION: The newer flow cell tips (Figure 4.12-5) appear slightly lower when in the correct position than do the older flow cell tips (Figure 4.12-6).

6. Place the tip at the center of the fluorescence pickup lens:
    a. Turn the flow cell horizontal adjustment as needed.
    b. Turn the flow cell vertical adjustment as needed.

Note: If the LED is turned on and the mirror is in place, a white dot appears as shown in the newer flow cell tip in Figure 4.12-5. If not, adjust the flow cell horizontal and flow cell vertical knobs. See Parabolic Mirror on Tip, Optional for more information.

Figure 4.12-5 New Flow Cell Body

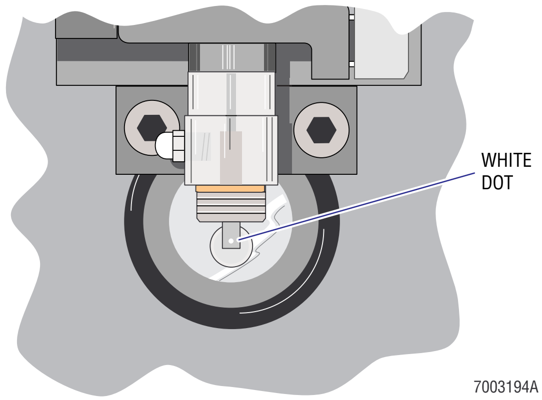
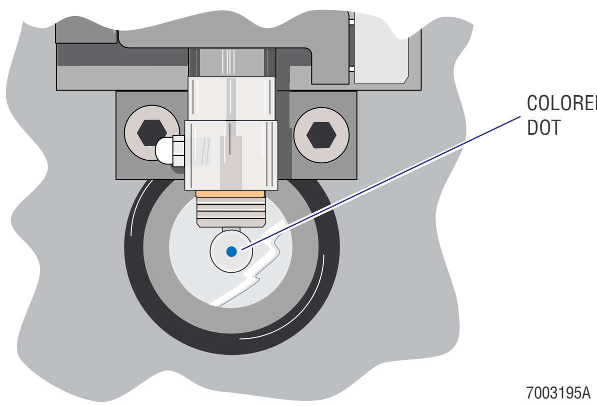


Figure 4.12-6 Old Flow Cell Body

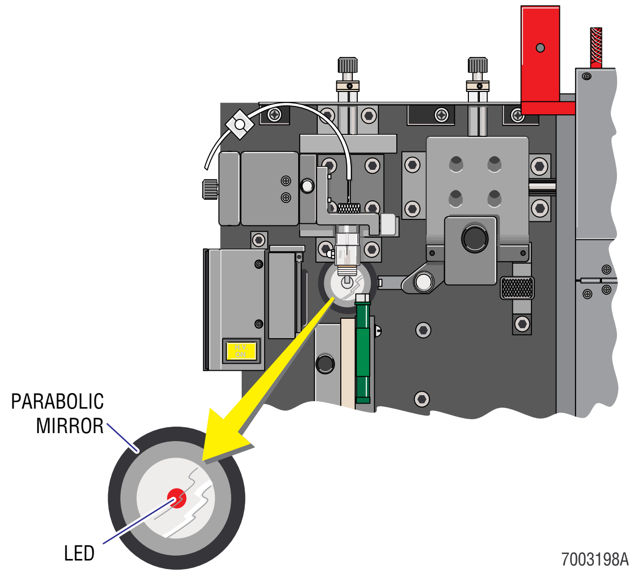


7. Do Laser Beam Alignment.

Parabolic Mirror on Tip, Optional

1. At the Cytometer:
   a. Select **Options**.
   b. Change **Align LED** to **Continuous**.
   c. Select **Main**.
2. Insert a mirror into the first filter slot on the right.The LED backlights the pinhole and provides an image marking the center of the assembly.
3. Close the laser shutter.
4. Center the LED image in the parabolic mirror. See Figure 4.12-7.
   - Adjust the flow cell vertical knob as needed.
   - Adjust the flow cell horizontal knob as needed.

Figure 4.12-7 Parabolic Mirror and LED

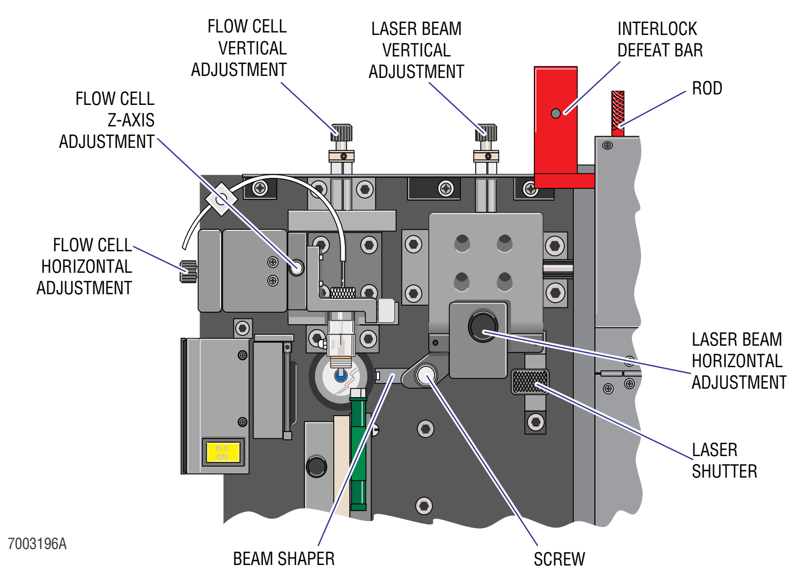


5. Turn off the LED.
6. Remove the mirror from the first slot on the right.
7. Do Laser Beam Alignment.

Laser Beam Alignment

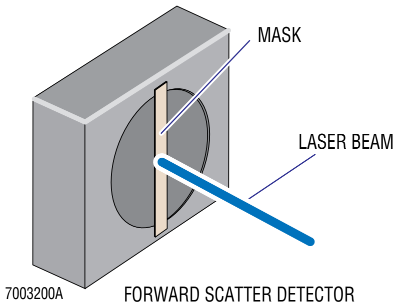
1. Press **SHEATH**.
2. Open the laser shutter. See Figure 4.12-8 for location.

Figure 4.12-8 Laser Shutter Location



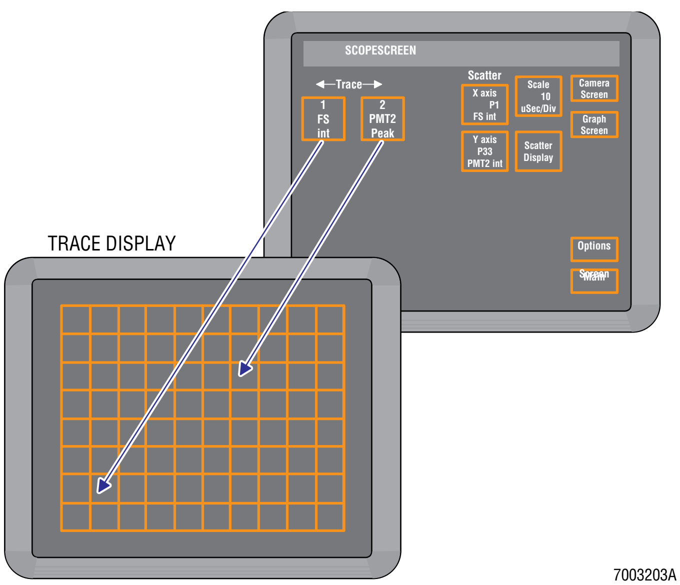
3. Center the laser beam vertically on the forward scatter detector mask by adjusting the laser beam vertical knob. See Figure 4.12-9.

Figure 4.12-9 Laser Beam Centered on Forward Scatter Detector Mask



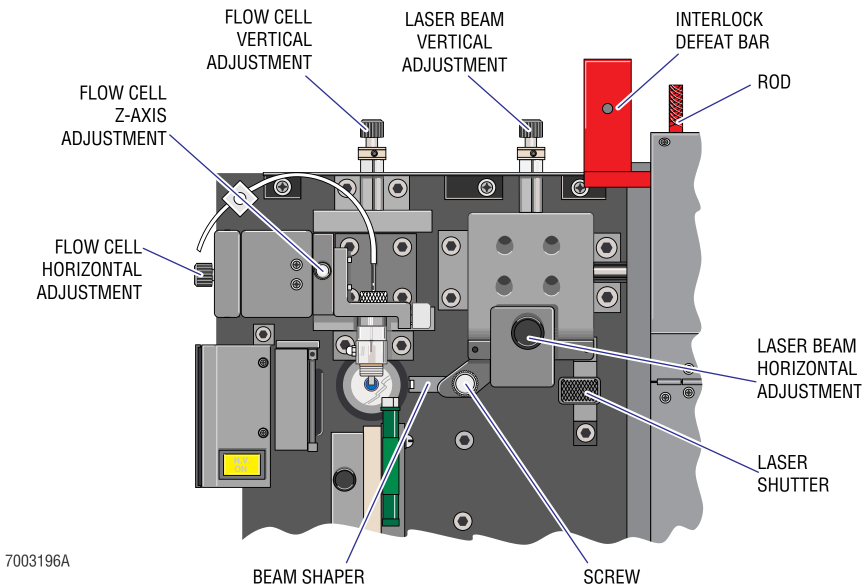
4. At the Cytometer:
   a. Turn the left scope knob clockwise.
   b. Select **Scope**.
5. As shown in Figure Figure 4.12-10, select:
   a. **Trace 1** and **FS Int**.
   b. **Trace 2** and **PMT2 Peak**.

Figure 4.12-10 Scope Screen Selections



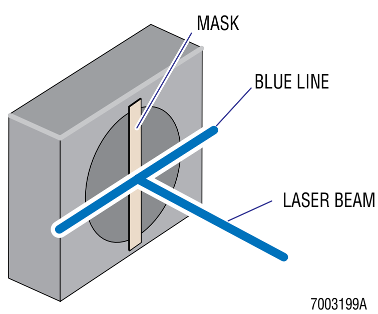
6. Place a sample of fluorospheres on the instrument and press **RUN**.
7. Center the beam on the absorber in front of the light scatter detector by adjusting the laser beam horizontal knob. See Figure Figure 4.12-11 for location.

Figure 4.12-11 Laser Beam Horizontal Adjustment Location



8. Slowly adjust the laser beam horizontal knob in one direction, and watch for the horizontal blue line or spot to appear, disappear, and reappear across the forward scatter detector.
9. Adjust the laser beam horizontal knob half way between the two horizontal blue line positions as shown in Figure 4.12-12.

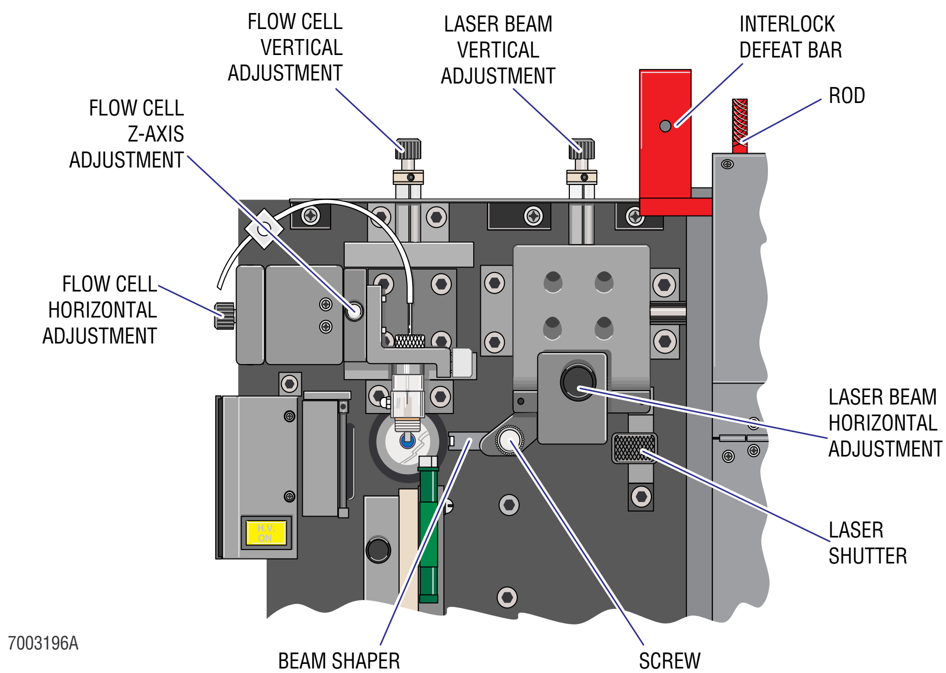
Figure 4.12-12 Laser Beam Centered Across Forward Scatter Detector



10. Verify that pulses appear when you scan between the horizontal blue line positions.
11. If no pulses appear, or if they do not appear to be normal:
    a. Check for a fluidics problem, such as bubbles, tube not sealed, and so forth.
    b. Verify that the flow cell tip is clean. Clean if needed.
    c. Verify that the forward scatter mask is all the way in position.

12. If pulses still do not appear, or if there is a lot of background noise, scattered light from the beam may be entering the sensor:
    a. Adjust the laser beam horizontal knob so the beam is in the center of the mask and all noise disappears from the scope.
    b. Adjust the flow cell Z-axis knob until good forward scatter pulses appear. See Figure 4.12-13 for knob location.
13. Maximize the forward scatter pulse by adjusting the flow cell Z-axis knob.
14. Fine tune for maximum forward scatter pulse height by adjusting the horizontal laser beam knob.
15. Do Optical Fine Tuning.

Figure 4.12-13 Flow Cell Z-Axis Knob Location

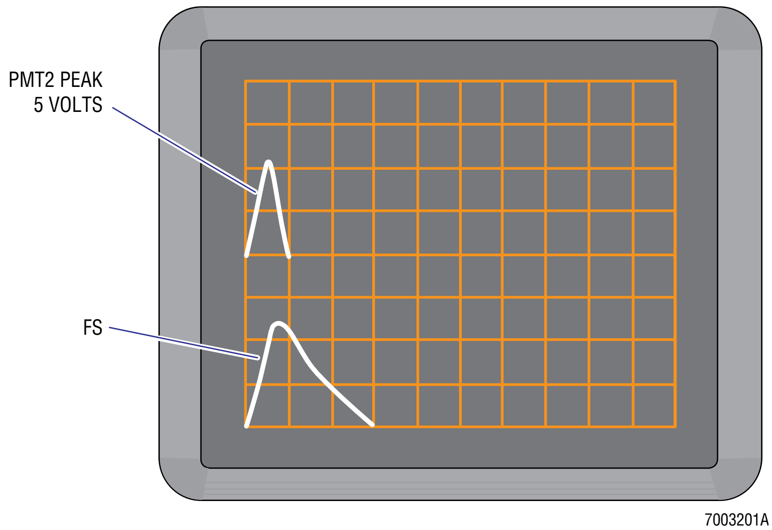


Optical Fine Tuning

Optical fine tuning is achieved through the use of fluorescence peak pulses. To properly optimize the system, the pulses should originate from a top PMT (reflected image), such as PMT 2, and the last PMT (straight-through image). If the optimal for one path is not optimal for the other, the adjustment is positioned between the two optimal positions.

1. At the Cytometer:
    a. Select **Main**.
    b. Adjust the PMT2 high voltage for a PMT2 peak pulse of about 5 V high. See Figure 4.12-14.

Figure 4.12-14 PMT2 High Voltage Setting



- If the PMT2 pulse does not appear or is very noisy, the flow cell may not be aligned enough to the center of the fluorescence pickup lens. Adjust the flow cell vertical and horizontal knobs until the pulse appears.
- The traces are set differently depending on if the last PMT is PMT4 or PMT5.
  - If you have a 4 PMT system, go to PMT4 System.
  - If you have a 5 PMT system, go to PMT5 System.

PMT4 System

ATTENTION: Risk of losing traces if the steps for fine tuning the PMT4 are not followed in exact sequence. Follow the sequence of steps to avoid losing the traces when setting up the scope.

1. At the Cytometer, select:
   a. **Trace 1** and **PMT2 Peak**.
   b. **Trace 2** and **PMT4 Peak**.
   c. **Main**.
2. Adjust PMT4 high voltage for PMT4 peak pulse of about 5 V. See Figure 4.12-15.
3. Obtain maximum pulse heights:
   a. Adjust the flow cell horizontal knob as needed.
   b. Adjust the flow cell vertical knob as needed.
   c. Adjust the laser beam vertical knob as needed.
   d. Adjust the fluorescence pickup lens (Figure 4.12-16) for simultaneous maximum pulse heights.
4. Repeat step 3 at least once.
5. Obtain minimum pulse widths by adjusting the focus knob, and collect new histograms and recheck the statistics. See Figure 4.12-17 for the knob location.

Figure 4.12-15 PMT4 High Voltage Setting

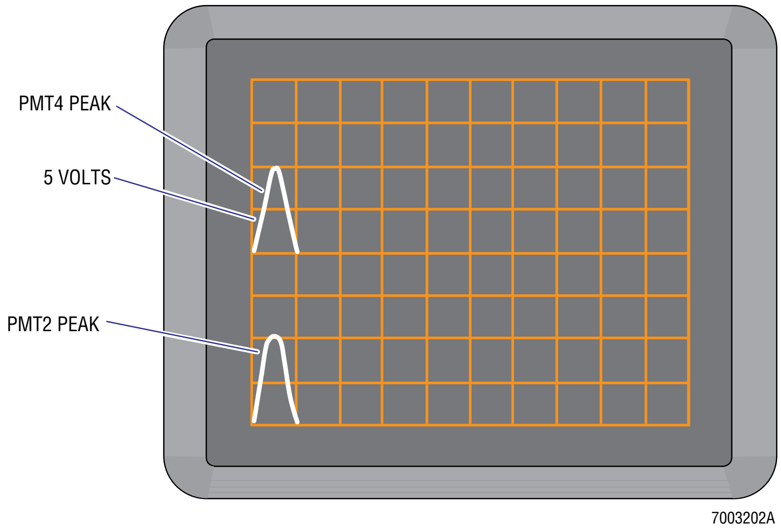


Figure 4.12-16 Fluorescence Pickup Lens

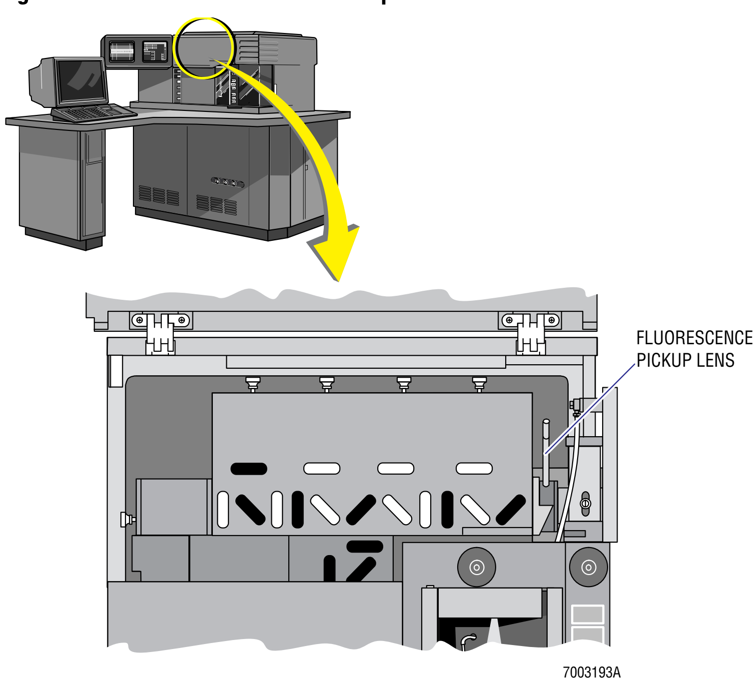
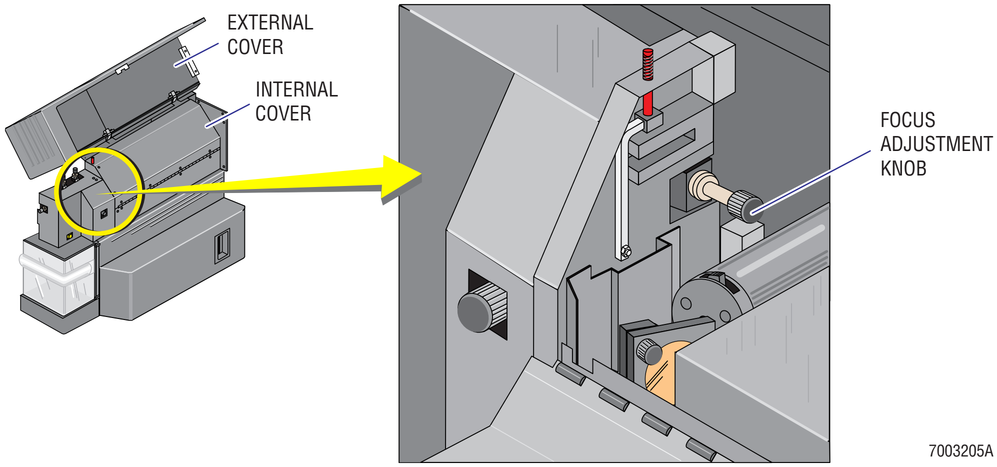


Figure 4.12-17 Focus Knob Location



PMT5 System

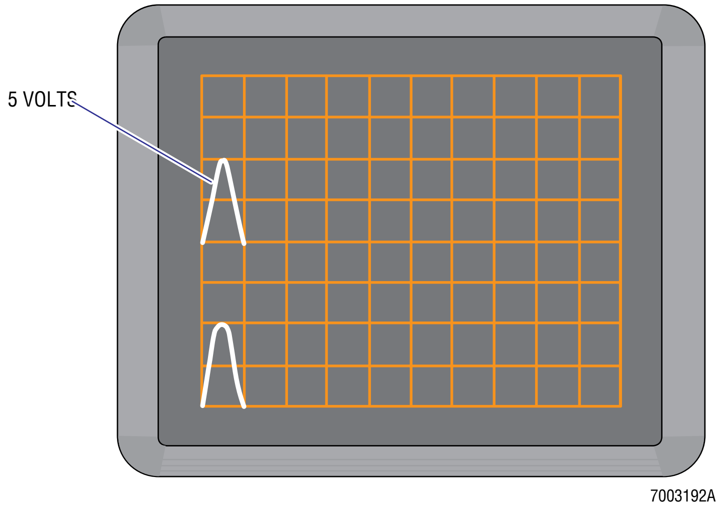
ATTENTION: Risk of losing traces. Follow the sequence of steps to avoid losing the traces when setting up the scope.

1. At the Cytometer, select:
   a. **Trace 1** and **PMT2 Peak**.
   b. **Trace 2** and **PARAM B**.
   c. **Main** ▸▸ **Options** ▸▸ **Gated Amp Assign** ▸▸ **PARAM B**, **AUX2 Peak.**
   d. Source box under AUX2 and PMT5.

   Note: PMT5 peak pulse now appears on the upper trace, trace 2.

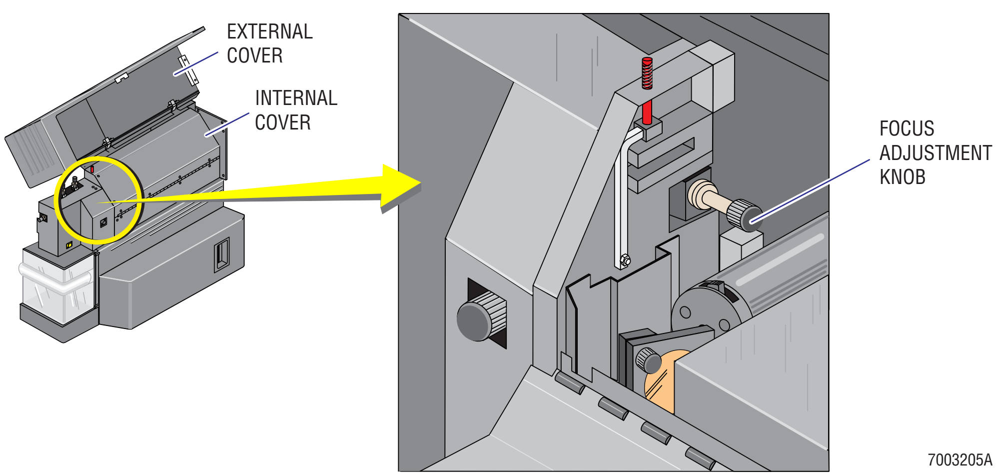
   e. **Main**.
2. Set the **AUX 2** peak gain to 10.
3. Adjust the **PMT5** high voltage for 5 V pulse height on trace 2, top trace. See Figure 4.12-18.

Figure 4.12-18 PMT5 High Voltage Setting



4. Obtain maximum pulse heights:
   a. Adjust the flow cell horizontal knob as needed.
   b. Adjust the flow cell vertical knob as needed.
   c. Adjust the laser beam vertical knob as needed.
   d. Adjust the fluorescence pickup lens for simultaneous maximum pulse heights.
5. Repeat step 4 at least once.
6. Obtain minimum pulse widths by adjusting the focus knob. See Figure 4.12-19 for the knob location.
7. Collect new histograms and recheck the statistics.

Figure 4.12-19 Focus Knob Location



4.13 EXTERNAL MEMORY CARD REPLACEMENT

Purpose

Use this procedure to either replace the 256K Memory card with the External Memory card or to replace a defective External Memory card. The circuitry on this External Memory card works effectively with the Cytometer CPU circuitry which minimizes the potential for a CPU lockup to occur.

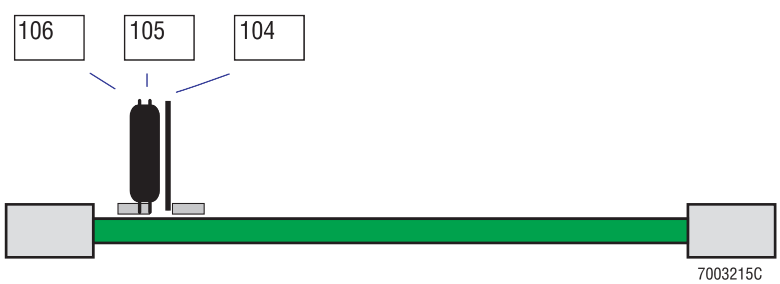
Tools/Supplies Needed

- ❑ External Memory card, PN 7000681
- ❑ Jumper, 2-position discrete wire, PN 2121023

Procedure

1. Remove the left panel to allow access to the Multibus card cage in the electronic pedestal.
2. Inspect the Multibus card cage to determine the type of memory card present - the older 256K Memory card or the newer External Memory card.
   a. If an External Memory card is present:
      1) Remove the defective External Memory card and set it aside.
      2) Go to step 3.
   b. If a 256K Memory card is present:
      1) Remove the 256K Memory card and set it aside.
      2) Locate and remove the CPU card.
      3) Install a jumper across pins 105 and 106 on the front, left area of the CPU card (Figure 4.13-1).

Figure 4.13-1 CPU Card, Jumper Locations



3. Install the new External Memory card in a free slot under the CPU card.
4. Power on the Cytometer.
5. Press and hold the reset switch for approximately 10 seconds until a tone is heard from the newly installed circuit card.
6. Reinstall the front, left panel back on the electronic pedestal.
7. Power on the Workstation.
8. Boot to the Elite software.
9. Press F9.

10. When the prompt to transfer the program into the Cytometer appears, select **Yes** to initiate memory transfer from the Workstation to the CPU. The number “1” appears on the Cytometer right monitor and begins to sequence as the files are transferred.
11. Wait while the number displayed on the right monitor sequences (or counts) from 1 to approximately 425. This process takes several minutes.
12. Under Heading 5.1, VERIFICATION INSPECTION PROCEDURE, complete the instructions under heading Operational Test - Cytometer Electronics, Optics, or Fluidics as written.

CONTENTS

5.1 VERIFICATION INSPECTION PROCEDURE

Verifying Instrument Performance Every Call

Cytometer

1. Ensure all fixed panels and covers are in place.
2. Ensure all moveable covers operate properly.
3. Inspect all fans:
   - Two on Multibus card cage
   - One above Gated Amp card cage
   - Two on right rear panel
   - One on left rear panel
4. Inspect sampling and collection areas for leakage and spillage. Clean and repair as needed.
5. Inspect flow cell area for saline spray deposits and corrosion. Clean and repair as needed.
6. Inspect air/water separator.

Workstation

1. Go to DOS directory.
2. Run SCANDISK on C:.

System Not Serviced Within Past 30 Days

1. Inspect and adjust compressor module as needed for correct pressures. Verify that the Compressor module is freestanding on the floor and does not touch the instrument.
2. Perform Powerup Valves at the Cytometer.
3. Set Sheath and Sample pressures to 13 psi.
4. At Valves screen, ensure that SET and READ pressures agree within ±0.05 psi.

   Note: If pressures do not agree, do Pneumatic Interface card calibration procedure.
5. Power up all lasers. Ensure blowers are operating.
6. Verify operation of pneumatic laser shutter.
7. Verify operation of mechanical laser shutter.
8. Verify operation of sample vial detect.

Operational Test - Cytometer Electronics, Optics, or Fluidics

Do this procedure if repairs were made affecting Acquisition, CV, or Analysis subsystem.

1. Create a protocol to acquire fluorospheres as follows (or use saved protocol suitable for acquiring fluorospheres):
   - Histogram 1, FALS vs. 90LS, stop at 5000
   - Histogram 2, FALS
   - Histogram 3, 90LS

- Histogram 4, Log GFL
- Histogram 5, GFL
- Histogram 6, Peak GFL
- FALS discriminator = 100, all others = OFF
- All gains =10; All HV= 400.

2. Select **Acquire** and ensure values transfer correctly to Cytometer.
3. Adjust settings as necessary to obtain good histograms.
4. Analyze for CV value. Ensure that the results are within specification for the fluorospheres used.
5. Verify operation of the oscilloscope.
6. Verify operation of the GRAPH display on the Cytometer.

Operational Test - Sorting Ability

If repairs were made affecting sorting electronics or mechanical elements, do Heading 4.2, SORT WAVEFORM VERIFICATION AND ADJUSTMENT PROCEDURE

Operational Test - Every Call

1. Have customer run one or more samples to verify operation.
2. Ensure that all customer concerns have been addressed.

TOF Verification

Purpose

This procedure describes how to verify TOF by creating a simple protocol to measure the FL Test pulse width using the PMT2 Peak signal.

Procedure

1. At the Workstation:
   a. Select **Acquisition ▸▸ Parameter**.
   b. Erase all parameters that may be present.
2. Verify that you are in the Create mode.
3. Select the following parameters in the Signal column
   a. **PMT2**
   b. **PMT2 Peak**
   c. **Time of Flight**
   d. **PMT2 Peak**.

   Note: The list of Parameters in the right column should read: *PMT2, PMT2 Peak, Time of Flight*. The Time of Flight box should contain *Param:PMT2 Peak*. If these items are not correct, repeat step 3.

4. At the Cytosettings screen:
   a. Set the Time of Flight-time base to 10.0 microseconds.
   b. Set the PMT2 Peak discriminator to 100, other channels to OFF.
5. At the Protocol screen, create single parameter histograms to acquire PMT2, PMT2 Peak, and Time of Flight.
6. Press [F9].
7. On the Cytometer touchscreen:
   a. Press **Options**.
   b. Under **Align LED**, select **Pulsed**,**100%**, **0.25 kHz**
8. Place the mirror filter holder in the dichroic (45 degree) slot below PMT1.
9. Remove the 525 nm filter below PMT2.
10. Adjust PMT2 HV and gain to obtain pulses on the Cytometer scope display for PMT2 Peak of about 5 V (two divisions) amplitude.
11. Note the Time of Flight blocks on the Cytometer Options screen. TOF should be on and Time base should be 10.0 microseconds.

    Note: Check your protocol if your readings are incorrect.
12. Verify that the protocol Cytosettings are set to **REC**, then press [F9] to restart acquisition. Observe the TOF histogram.
13. Adjust the Beam Width Normalization control to shift the distribution until the histogram mean channel corresponds to the PMT2 Peak pulse width (estimate based on Cytometer scope display; width of pulse at 15% above baseline).

    For example: For an observed pulse width of 6 microseconds and a TOF time base of 10 microseconds, the histogram mean channel is:

    $$\frac{6}{10} \times 1024 = 614.6$$

5.2 PREVENTIVE MAINTENANCE (PMI) PROCEDURE

Purpose

The PMI procedure is intended to help prevent failure of the Elite. In this procedure, you may be required to replace a worn component, clean the system, or make necessary adjustments to keep the Elite operating smoothly. This schedule is only a recommendation and may vary with instrument usage and environment.

Tools/Supplies Needed

- ❑ Elite PMI Kit, PN 6913419-0
- ❑ Brass T-fittings, PN 6216129-9
- ❑ Tubing connecting the T-fittings, PN 3202039-9
- ❑ Waste carrying tubing, PN 3213194-8 (PN 3202039-9 on older systems)
- ❑ Hydrophobic filter round disk type, 0.3 µ, PN 6232143-1
- ❑ Halogen lamp, PN 3908025-7
- ❑ Metal holder clamps for both sheath and waste filters, PN 2838068-2, PN 3203027-1
- ❑ Sheath filter interconnections, PN 6232472-4, PN 6232475-9, PN 6232522-4
- ❑ Sheath filter, PN 6232473-2
- ❑ Waste filter, PN 6232489-9
- ❑ Vial cap, PN 6856967-2
- ❑ Check valves in the waste lines, PN 6214106-9, PN 6801536-7
- ❑ Male and female waste tank connectors, PN 6232429-5, PN 6232452-0, PN 6232546-1, PN 6232547-0
- ❑ Sheath tank cap O-ring, PN 2523451-1

Before You Begin

1. Inspect the instrument for operational problems, and correct if found.
2. Do the appropriate portion(s) of the VIP under Heading 5.1, VERIFICATION INSPECTION PROCEDURE to verify operation of the repaired component(s).

Procedure

1. Ensure that the kit is complete by comparing the contents with the packing list
2. Turn the unit OFF.
3. Open pneumatics covers.
4. Replace all the brass T-fittings.
5. Replace all tubing that connects the T-fittings.
6. Replace all remaining waste-carrying tubing.
7. Replace hydrophobic filter, round disk type, 0.3 µ located on top of valve bracket assembly.

8. Replace the halogen lamp.

   Note: The used lamp should be marked as such and left with the operator as an emergency spare.

9. Replace the metal holder clamps for both the sheath and waste filters.
10. Replace the sheath filter interconnections.
11. Replace the sheath filter.
12. Replace the waste filter.
13. Replace the vial cap.
14. Replace all check valves in the waste lines.
15. Replace the male and female waste tank connectors.
16. Replace the sheath tank cap O-ring.
17. Inspect and clean:
    a. Beam shaping optics
    b. Filters
    c. Mirrors.
18. Replace all optical filters used in the filter housing every 5 years.
19. Inspect and clean flow cell and fluorescent pickup lens assembly.
20. Power up the unit and do the optical alignment procedure.
21. At the DOS prompt, type `DEFRAG` and press .

    Note: Units with older MS-DOS may not have the programs referred to in steps 21 through 25. If not, use the Troubleshooter Software.

22. If there are any problems, follow the DEFRAG instructions.
23. Reboot the Elite.
24. Do Heading 5.1, VERIFICATION INSPECTION PROCEDURE.

CONTENTS 6

6.1 SCHEMATICS REQUIRED

This chapter contains a list of the engineering schematics you need for troubleshooting the Elite flow cytometer.

Electronic (.pdf) files of these schematics are available on a separate CD-ROM in the Service Resource Kit (SRK) and in a Lotus Notes® database. The schematics in the SRK are the latest revisions available at the time the SRK is released. For copies of schematics released between revisions of the SRK, check the Lotus Notes database. It will always have the most current revisions.

Note: Depending on the configurations of this instrument in the field, more than one revision of a schematic can be valid.

If you want to include schematics in the printed version of this manual, make printouts of the electronic files and insert them at the end of this chapter.

Most Frequently Used Schematics

Gated Amp Interconnect	PN 6319353
Pneumatic/Hydraulic Layout	PN 6320201
Interconnect Diagram Laser Interlock	PN 6320234
Pneumatic/Hydraulic Layout Analyzer	PN 6321140

Additional Schematics for All Systems

Acquisition Card Cage (Lower)

Bitmap And Sort Decision*	PN 6319569
Data Acquisition Backplane	PN 6319561
Data Lister Out R	PN 6319520
Interface and Scaler R	PN 6319535
MUX & Scope Interface “R”	PN 6319541
Peak ADC PSH Card ESP†	PN 6320856
PRISM & Sort Window Test*	PN 6319570
Pulse Gen & Clock	PN 6320194
Pulse Pileup Det/TOF 2 ESP†	PN 6321443
Quad Peak Sense & Hold	PN 6319559
Sort Delay R-3 ESP*†	PN 6321511
Sort Oscillator R-2 ESP*†	PN 6321026
Sort Output R*	PN 6319584
Sort Transistor card	PN 6317927

** indicates not used in Analyzer system.*
† ESP indicates new or changed for the ESP system.

Gated Amp Card Cage (Upper)

3 PMT Sub SW-R	PN 320686
Dual FL Switch R card	PN 6320687
Gated Amp Backplane	PN 6319579
Gated Amp Control R-3 ESP†	PN 6320758
Peak Scatter MUX SW-R	PN 6320829
SCAT/CV SW-R	PN 6320688
Sensor Interface RP	PN 6319759

† ESP indicates new or changed for the ESP system.

Multibus Card Cage

Camera Interface R*	PN 6319828
DT Interface R	PN 6319006
DIGI Scope	PN 6319024
Dual CRT Controller	PN 6319472
Dual Laser Controller	PN 6319732
Extended Memory	PN 6323668
Serial I/O C	PN 6317988

** indicates not used in Analyzer system.*

Interconnect Diagrams

Interconnect Diagram AC L/N area	PN 6320236
Interconnect Diagram AC RH area	PN 6320233
Interconnect Diagram DC 400W Switcher	PN 6320235
Interconnect Diagram DC Distribution	PN 6320289
Interconnect Diagram DC Sensing area	PN 6320238

Other

±36 VDC Power Supply board*	PN 6319714
'A' board of Lister AT	PN 6319846
Analyzer High Voltage DAC	PN 6319272
'B' board of Lister AT	PN 6319850
HV DAC/PMT backplane	PN 6319296
KIR3	PN 6319727
Motor Controller R*	PN 6319855
Peak Scatter Sensor card	PN 6320547
Pinhole LED Driver	PN 6320016
PMT Connector Board R	PN 6319789
Pneumatic Interface R2	PN 6320927
Pump Motor Det (tube sensor)	PN 6319305
Sort HV Deflection Supply*	PN 6320917
Switch & Indicator R	PN 6319807

** indicates not used in Analyzer system.*

Additional Schematics for Analyzer Systems

Analyzer Interface	PN 6321014

Additional Schematics for Autoclone Sorting Option Systems

Autoclone Sorting Option card	PN 6320579
Interconnect	PN 6320653
Position board	PN 6320652

Additional Schematics for Gated Amp Systems

7 Microsecond Delay R	PN 6319615
PMT Gated Amp R	PN 6319342
Quad 20/40/60 Microsecond Delay R	PN 6319749
SCAT/AUX Gated Amp R	PN 6319345

Additional Schematics for Non-Current Production Systems

Pneumatic Interface R (before low bleed regulators)	PN 6319758
Scatter Sensor board	PN 6319728
Sensor Interface R (before Panelyzer)	PN 6319759

Additional Schematics for Non-ESP Systems

ADC & PSH Control	PN 6319509
Gated Amp Control R2	PN 6320143
Sort Delay R	PN 6319580
Sort Oscillator R	PN 6319576

Additional Schematics for Non-Switch Amp, Jumper Configured Cards Systems

3 PMT Sub Amp R	PN 6320138
Dual FL Amp R	PN 6320137
SCAT/CV Amp R	PN 6320136

ILLUSTRATIONS

TABLES

7.1 ISOLATING THE PROBLEM AREA

Purpose

This is a general procedure for isolating a problem area. The troubleshooting charts and methods in this section are examples of recommended troubleshooting approaches for use with common problem areas. Refer to the Special Procedures and Troubleshooting manual for further information.

Tools/Supplies Needed

- ❑ None.

Procedure

1. Do startups, alignment check, or other symptom checks to obtain a clear symptom.
2. Use the symptom to isolate the problem area.
3. Check the problem area for the types of problems shown in Table 7.1-1.

Table 7.1-1 Isolating Problem Areas

Problem Area(s)	Possible Causes
Biological sample	Bad reagents Poor preparation Abnormal sample
Electronics	Adjustment Poor connection Defective components
Filters	Wrong filter Dirty filter Defective filter
Fluidics (sheath, sample or waste)	Pinch Plug Leak Defective component
Laser(s)	No beam No power Current too high
Optics	Alignment
Software	Improper use Power problem Software bug

General Troubleshooting

Most problems that occur with the system are fluidics-related, and some of the fluidic problems result in the same symptoms noted in the Optics section of Table 7.1-2. Always check fluidics before attempting an alignment procedure. See Heading 7.3, TROUBLESHOOTING FLUIDIC PROBLEMS for details on fluidic troubleshooting.

Aside from fluidics, other troubleshooting may be required. See Tables 7.1-2 through 7.1-8:.

- Table 7.1-2, Autoclone Sorting Option, General Troubleshooting Information
- Table 7.1-3, General Troubleshooting Information
- Table 7.1-4, Lasers, General Troubleshooting Information
- Table 7.1-5, Optics*, General Troubleshooting Informationx
- Table 7.1-6, Electronics, General Troubleshooting Information
- Table 7.1-7, Software, General Troubleshooting Information
- Table 7.1-8, Power Supplies, General Troubleshooting Information.

Table 7.1-2 Autoclone Sorting Option, General Troubleshooting Information

Problem	Probable Cause	Corrective Action
No sheath flow.	Clogged flow cell.	1. Press **CLEAR** if sheath is fully clogged. For partial clogs, press **SHEATH** and **VACUUM**. 2. If still clogged, brush tip with a camel hair brush, and press **SHEATH** and **VACUUM**. 3. If still clogged, remove flow cell tip and force liquid or air through it.
Stream is deflected or unstable.	Partially clogged flow cell.	1. Press **SHEATH** and **VACUUM**. 2. If still clogged, brush tip with a camel hair brush, and press **SHEATH** and **VACUUM**. 3. If still clogged, remove flow cell tip and force liquid or air through tip.
Low data rate	Partially clogged flow cell.	1. Press **SHEATH** and **VACUUM**. 2. If still clogged, brush tip with a camel hair brush, and press **SHEATH** and **VACUUM**. 3. If still clogged, remove flow cell tip and force liquid or air through it.
	Bubbles	Press **DEBUBBLE**.
	Tube not properly sealed.	Push cap into tube.
	Pinched sample line.	Work the tubing until pinch is removed.
	Low sample pressure	Slightly increase flow rate.

Table 7.1-2 Autoclone Sorting Option, General Troubleshooting Information *(Continued)*

High data rate.	Sheath tank not properly sealed.	Reseal sheath tank.
	Discriminator too low.	Set to higher value.
	Extraneous light entering sensor.	Check mask and alignment; check for external light sources.
Long pulse rise time.	Bubbles or debris in flow cell tip.	Press **DEBUBBLE**. OR Press **SHEATH** and **VACUUM**, then press **DEBUBBLE**.
Alignment check. Shifted mean channel or high CVs.	Same as all probable causes listed above.	Press **DEBUBBLE**. OR Press **SHEATH** and **VACUUM**, then press **DEBUBBLE**.
	Horizontal beam, flow cell, and/or vertical beam not properly aligned.	Do alignment procedure.
FS pulse: shifted mean, high CVs, fluorescence okay	Dirty flow cell.	Clean flow cell with lens cleaning paper, cotton swab, and optical grade methanol.
	FS detector mask not positioned properly.	Reposition mask.
Drain(s) overflow.	Waste tank not sealed.	Reseal tank.
	Quick-disconnects not connected.	Reconnect quick-disconnects.
	Pinched waste line.	Follow drain line and remove pinch.
	Weak vacuum pump.	
Acquisition appears on the cytometer screen but not on the workstation monitor.	Problem with Lister card or with parallel cable.	

Table 7.1-3 General Troubleshooting Information

Problem	Probable Cause	Corrective Action
Autoclone Sorting Option		
Noisy movement	Incorrect motor speed	Adjust motor speed.
	Interference or friction	Locate and correct.
Unit hits limit switch during calibration	Optical sensor	1. Check optical sensor and encoding strip for dust or contamination. Wipe clean as needed. 2. Verify operation of sensor by manually moving the arm assembly while reading the marker box on the Autoclone Sorting Option Control screen.

Table 7.1-3 General Troubleshooting Information *(Continued)*

Problem	Probable Cause	Corrective Action
	Offsets too much	Realign the mechanism to center the uncharged stream in the wells with zero offsets.
Tray does not rotate exactly 180 degrees during operation.	Setscrew that secures the tray holder to the stepper motor shaft is loose.	Tighten the setscrew through the upper hole in the front of the arm.
	Rotation speed is incorrect.	Adjust speed as needed to obtain smooth rotation without overshoot.
Incorrect movement	LED markers do not appear as expected	1. Check optical sensor and encoding strip for dust or contamination. Wipe clean as needed. 2. Verify operation of sensor by manually moving the arm assembly while reading the marker box on the Autoclone Sorting Option Control screen. 3. Observe the LED markers on the Autoclone Sorting Option card: • If the markers indicate the Autoclone Sorting Option arm position correctly, then the problem is with communication between the Autoclone Sorting Option card and the Cytometer computer. Check the ribbon cable that connects the Autoclone Sorting Option card to the host system. • If the markers do not indicate the Autoclone Sorting Option arm position correctly, then check the following: ▸ Flex cable ▸ Position Detect card ▸ Interconnect card ▸ Main cable ▸ Autoclone Sorting Option card
No movement	No power to motors	Check all power supplies. Adjust or replace as needed.
Waste catcher (beak) overflows	Insufficient vacuum from compressor	Check compressor output. Repair or replace as needed.
	Vacuum leak or obstruction	Locate and repair
Waste catcher does not extend	Corroded cylinder	Replace cylinder
	No pressure to cylinder	Check tubing and solenoid valves. Replace as needed.

Table 7.1-3 General Troubleshooting Information *(Continued)*

Problem	Probable Cause	Corrective Action
Incorrect number of beads in well	Host instrument not correctly adjusted for optimal sorting.	Do Sort Setup procedure in Operator's Guide.
	Sorted stream is misaligned.	1. Adjust stream alignment and deflection so sorted stream does not strike water catcher 2. Ensure plates are clean and dry.

Table 7.1-4 Lasers, General Troubleshooting Information

Problem	Probable Cause	Corrective Action
Laser fails to turn ON.	Interlock keys	
Laser current is high on Cytometer Control Screen.	Dirty air intake filter.	Clean air intake filter.

Table 7.1-5 Optics*, General Troubleshooting Information

Problem	Probable	Corrective Action
Poor CVs with DNA-Check or IMMUNO- CHECK beads.*	Optics are not aligned.	Do Heading 4.1, OPTICAL ALIGNMENT PROCEDURE.
Shifted mean channels.*	Optics are not aligned.	Do Heading 4.1, OPTICAL ALIGNMENT PROCEDURE.
No data.*	Optics are not aligned.	Do Heading 4.1, OPTICAL ALIGNMENT PROCEDURE.
No pulses appear, or pulses do not appear normal.*	Optics are not aligned.	Do Heading 4.1, OPTICAL ALIGNMENT PROCEDURE.
	Flow cell tip is dirty.	Clean the flow cell tip.
	Forward scatter mask is not properly in place.	Verify that the forward scatter mask is properly seated.
	Scattered light from the beam may be entering the sensor.	1. Adjust the beam horizontal to the center of the mask until all noise, if any, disappears from the scope. 2. Adjust the flow cell Z-axis until good forward scatter pulses appear. 3. Roughly maximize the forward scatter pulse with the flow cell Z-axis.
PMT2 signals low and require increased HV to be seen properly.	Flow cell is not aligned to the center of the fluorescence pickup lens.	Adjust the flow cell vertical and or horizontal until the pulse increases.

Table 7.1-5 Optics*, General Troubleshooting Information *(Continued)*

Problem	**Probable**	**Corrective Action**
Traces were lost while setting up the scope.	Pulse selection for Trace 1 is too low.	1. Select **FS** for Trace 1 initially to establish pulses. 2. Check desired Trace 1 pulse on Trace 2 and adjust to a reasonable pulse height before displaying on Trace 1. 3. Select traces in order: • With a 4PMT system, select **Trace 1** then **PMT2 Peak**; and **Trace 2** and **PMT4 Peak**. • With a 5PMT system, select **Trace 1** then **PMT2 Peak**; and **Trace 2** then **PARAMB(1)**.

Most problems that occur with the instrument are fluidic - related, and some of the fluidic problems result in the same symptoms noted in this table.* ***Always check fluidics before doing an alignment procedure.*

Table 7.1-6 Electronics, General Troubleshooting Information

Problem	**Probable Cause**	**Corrective Action**
System locks up.	Power problems.	Power down the system; wait 30 seconds, then power up.

Table 7.1-7 Software, General Troubleshooting Information

Problem	**Probable Cause**	**Corrective Action**
Touch Screen locks up.	Power problems.	Power down the system; wait 30 seconds, then power up.
Workstation locks up.	Power problems.	Press Ctrl+Alt+Delete to reboot the computer. OR Turn computer off; wait 30 seconds, then turn computer on.

Table 7.1-8 Power Supplies, General Troubleshooting Information

Problem	**Probable Cause**	**Corrective Action**
No sort deflection.	Problem with 3000 V deflection supply.	Check and/or replace.
	First alligator clip is not attached to the insertion rod.	Attach alligator clip properly.
No stream undulation or deflection.	Problem with 36 V and 90 V power supplies.	Measure power supply and replace if defective. See Heading 4.4, POWER SUPPLIES: MEASUREMENT AND ADJUSTMENT.
Camera stops moving; camera cannot be adjusted; incorrect readings on the Control Screen.	Problem with 15 V, 5A power supply.	Measure power supply and replace if defective. See Heading 4.4, POWER SUPPLIES: MEASUREMENT AND ADJUSTMENT
PMT high voltages cannot be adjusted.	Problem with 15 V, 3.4A power supply.	Measure power supply and replace if defective. See Heading 4.4, POWER SUPPLIES: MEASUREMENT AND ADJUSTMENT

Table 7.1-8 Power Supplies, General Troubleshooting Information *(Continued)*

Problem	Probable Cause	Corrective Action
No signals.	Problem with 15 V, 0.25A power supply.	Measure power supply and replace if defective. See Heading 4.4, POWER SUPPLIES: MEASUREMENT AND ADJUSTMENT
Unable to make screen selections; computer locks up.	Problem with 5 V power supply.	Measure power supply and replace if defective. See Heading 4.4, POWER SUPPLIES: MEASUREMENT AND ADJUSTMENT
No image on CRTs; wavy lines on displays; or CPU problems.	Problem with 12 V power supply.	Measure power supply and replace if defective. See Heading 4.4, POWER SUPPLIES: MEASUREMENT AND ADJUSTMENT
Solenoids do not fire.	Problem with 24 V Power Supply.	Measure power supply and replace if defective. See Heading 4.4, POWER SUPPLIES: MEASUREMENT AND ADJUSTMENT

7.2 TROUBLESHOOTING THE ACQUISITION SUBSYSTEM

Purpose

Do this procedure to troubleshoot the Acquisition subsystem.

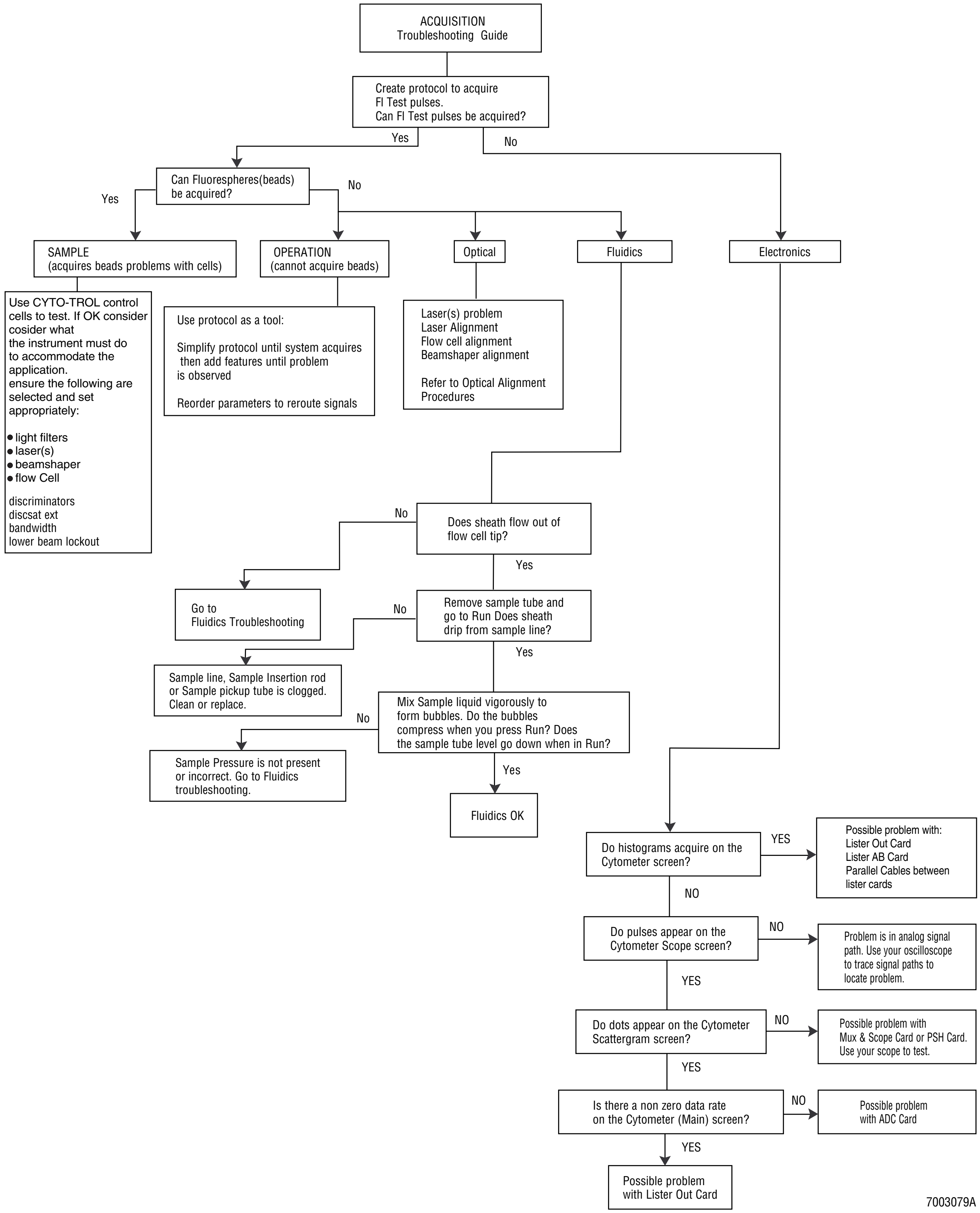
Tools/Supplies Needed

- ❑ None.

Procedure

1. Use Figure 7.2-1, Acquisition Troubleshooting Flowchart as a guide when troubleshooting the Acquisition subsystem
2. If the problem persists, see Acquisition Troubleshooting Methods for additional instructions.

Figure 7.2-1 Acquisition Troubleshooting Flowchart



Acquisition Troubleshooting Methods

Subtraction (Color Compensation)

Rule out electronic problems by testing the electronic subtraction circuits for proper operation. If no electronic problem is detected, focus on instrument setup, filters and sample preparation.

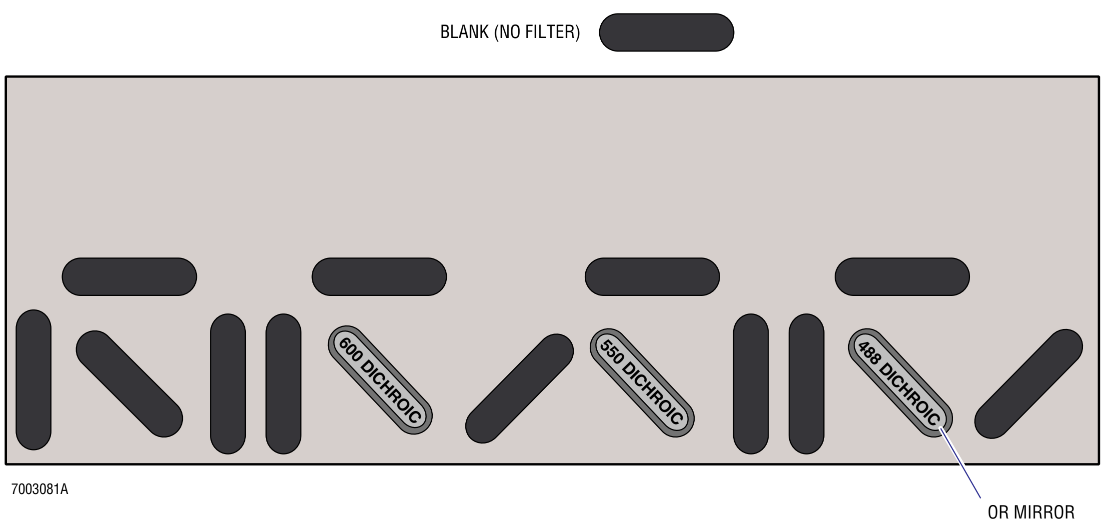
This test can be performed for the other PMTs by moving the filters as needed. Note that the test is always done with the PEAK PMT signal. Although subtraction is also seen on the Integral and log histograms, the reduction does not relate directly to the subtraction percent (especially for log),

Do the electronic test by providing the same pulse to two PMTs and then observe the histograms as subtraction is used. In this example, PMT2 and PMT3 subtraction is tested. You can use the same procedure with appropriate adjustments to test all subtraction combinations.

PMT 3 - PMT 2 Compensation

1. At the Cytometer, power down Sheath, Vacuum and Valves.
2. Turn lasers OFF.
3. Configure the filters as shown in Figure 7.2-2.

Figure 7.2-2 Filter Configuration



4. At the Cytometer Options screen, set **Align LED** to **Pulsed**, **0.25kHz** and **100%**.
5. Create a protocol to acquire PMT2 peak and PMT3 peak histograms as follows:

   Parameters - PMT2 Peak, PMT3 Peak
   Histograms - PMT2 Peak, PMT3 Peak, PMT2 x PMT3 Peak
   PMT2 discriminator = 100
   PMT2 gain = PMT3 gain = 20
   All color compensation to 0% (initial)

6. Begin acquisition and adjust PMT2 and PMT3 HV so the mean channel of both histograms is at channel 512
7. Test subtraction:
   a. Set **PMT3 - PMT2** (subtract PMT2 from PMT3) to 25%.
   b. Acquire and observe that PMT3 mean is reduced by 25% (512 - 0.25*512 = 384).

   Note: Mean for PMT3 should be 384 ±15 channels.

   c. Set **PMT3 - PMT2** (subtract PMT2 from PMT3) to 50%.
   d. Acquire and observe that PMT3 mean is reduced by 50%.

   Note: Mean for PMT3 should drop to 256 ±15 channels.

Log Amplifiers

If there is no log amp output, or if the output is incorrect:

1. At the amplifier cards, either on the backplane or at the card input, measure the voltage to ensure that the 15 V power supplies yield ±15.00 V.
   a. If not, adjust the power supply.
   b. If adjusting the power supply is not effective, replace the power supply.
2. Disconnect the amplifier input.
3. Locate the correct output connection.

   Note: Refer to Chapter Heading SCHEMATICS for schematics and interconnect diagrams, or read the card edge to locate the correct output connection.
4. Measure the amplifier output; it should be negative at least 100 mVdc.

   Note: If the card has 0 output or positive output, replace the card.

7.3 TROUBLESHOOTING FLUIDIC PROBLEMS

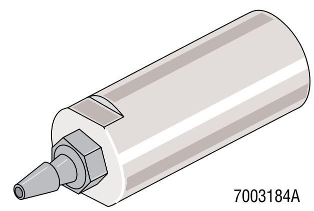
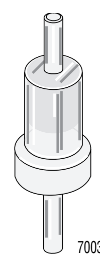
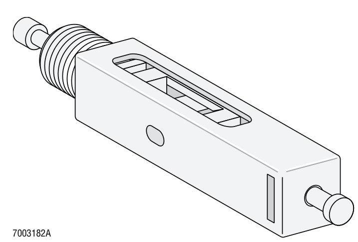
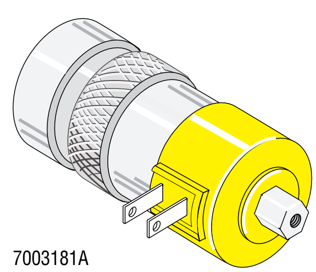
Purpose

Do this procedure to detect and repair fluidic problems. Generally, fluidic problems are categorized into three groups:

- Leaks - where fluid (air or liquid) escapes from the system.
- Blockages - where fluid (air or liquid) flow is restricted.
- Improper functions - where fluidic control components, such as valves and solenoids, do not function properly.

See Table 7.3-1 for fluidic components. Use Figure 7.3-1 as a guide for general fluidic troubleshooting guidelines.

Table 7.3-1 Fluidic Components

Component	Function	Illustration
Pilot actuator	Air pressure moves a piston inside the actuator, which then moves something else, such as a pinch valve.	 7003184A
Check valve	Permits flow in one direction only.	 7003183A
Pinch valve	Controls the path of fluid flow.	 7003182A
Solenoid	Controls the pressurization of other components.	 7003181A

Tools/Supplies Needed

- ❑ Pressure and Vacuum gauge (accurate to.01 psi, range 0 to 30psi)
- ❑ Hemostats
- ❑ DVM

Air Leaks

Sheath Air Leak Testing

1. At the Cytometer, select **Powerup Valves** and wait for sheath pressure to stabilize.

   Note: At this point the air in the sheath tank is pressurized to the Sheath pressure setting.

2. Press **VACUUM** and listen to the sheath regulator.

   - If you detect a leak, go to step 3.

     Note: Since no liquid is leaving the Sheath Tank, no additional air should be entering the tank. If you hear a buzzing sound coming from the regulator, this means air is being supplied to compensate for a leak.

   - If you do not detect a leak, go to step 4.

3. Apply soapy water to the bottle top and connections to locate the source of the air leak.If you cannot find the leak:

   a. Tee your pressure gauge to the regulator output, and clamp the line between the regulator and your gauge. Watch for the pressure to drop. A steady drop indicates a leak.

   b. If you still cannot find the leak, use two clamps: one between the regulator and gauge and the other to clamp each leg, in turn, away from the gauge.

4. Activate the Sheath and listen to the sheath regulator.

   Note: It should cycle every 10 to 60 seconds to compensate for liquid flow to the flow cell. If the regulator does not cycle, there may be a leak or internal problem, which requires the regulator to be replaced.

Liquid Leak Testing

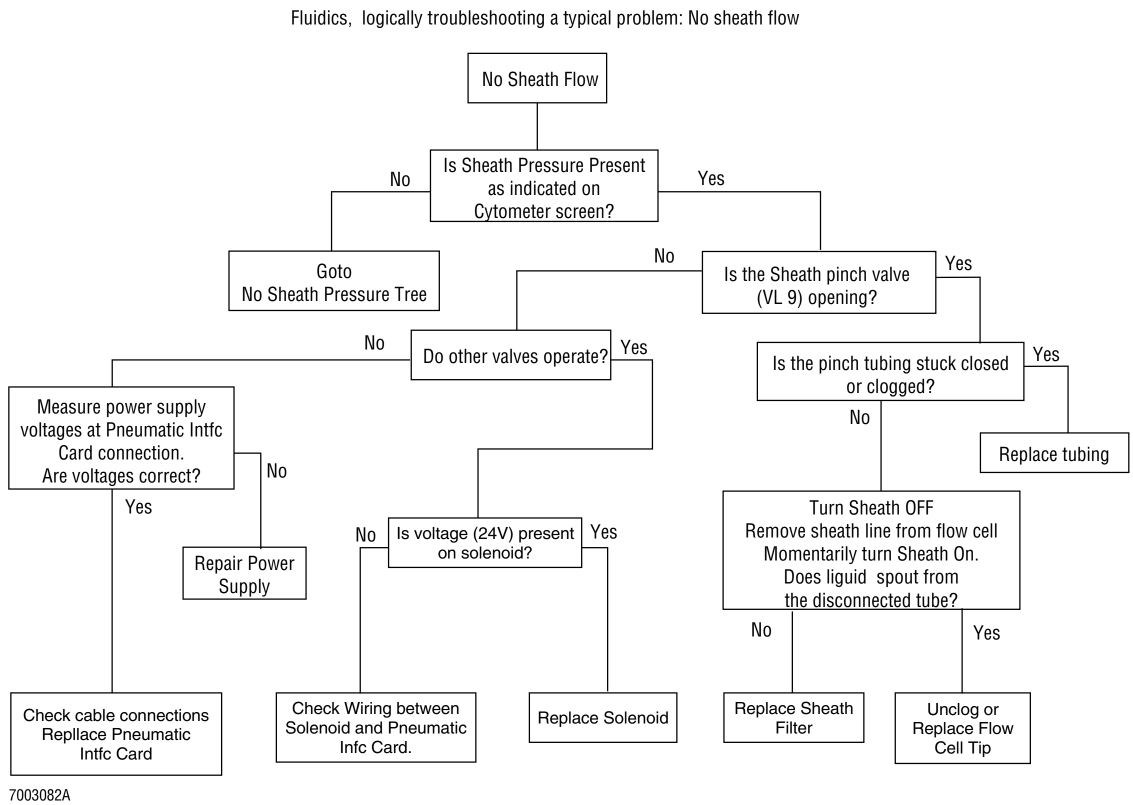
Sheath Liquid Leak Testing

1. Look for salt residue or rust around fittings and connections at the Sheath Tank.

   Note: Salt residue and rust indicate present or past leakage. Repair the leak and clean away all deposits.

2. Inspect feed-thru fittings for small leaks. Replace the fittings if you detect a leak.

Figure 7.3-1 Fluidics Troubleshooting Information



7.4 TROUBLESHOOTING PRESSURE CONTROL PROBLEMS

Purpose

Do this procedure to troubleshoot sheath and sample pressures.

Tools/Supplies Needed

- ❑ DVM
- ❑ Pressure gauge.

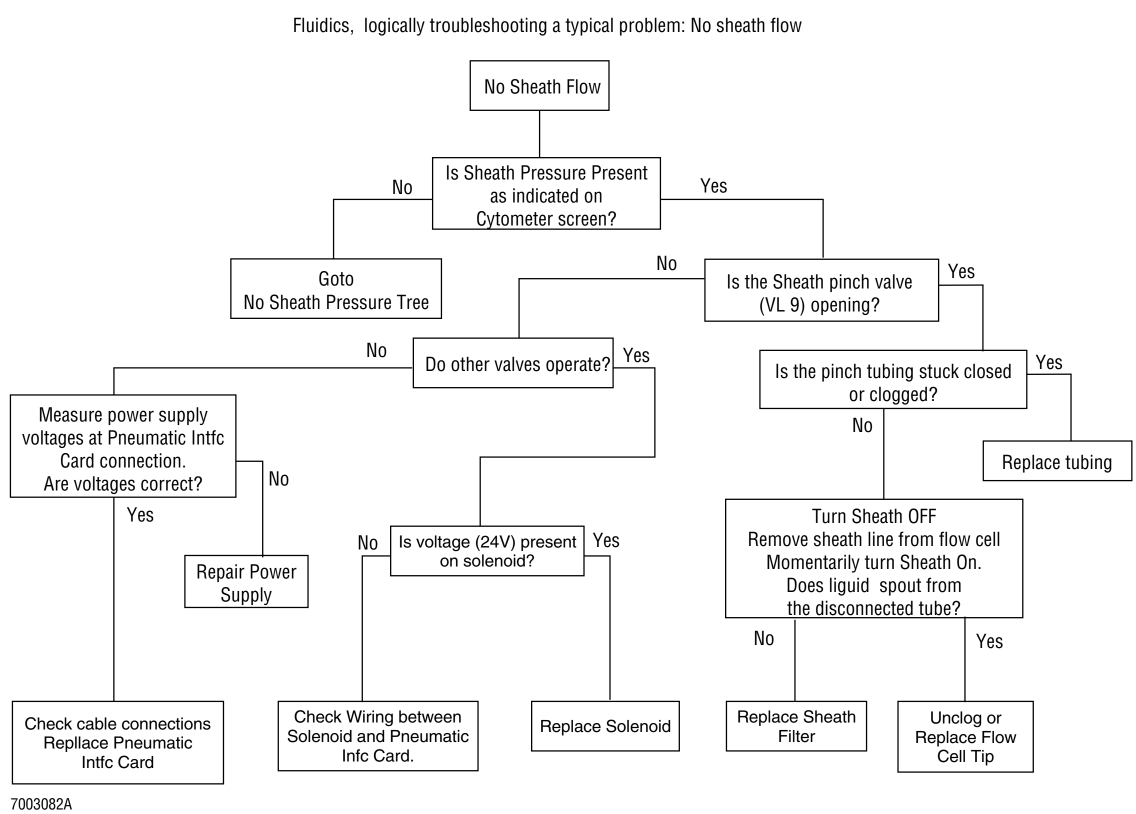
Procedure

Sheath Pressure Problems

Troubleshooting this system can be difficult because of the feedback from the in-line liquid pressure sensor. This can be turned off with the jumper on the Pneumatic Interface card to troubleshoot. Figure 7.4-1 shows how to troubleshoot for sheath flow.

Note: You should find that little if any adjustment is needed. In this configuration, the system uses the liquid pressure reading to adjust the sheath air pressure to maintain constant liquid pressure. Test this by raising or lowering the sheath bottle. You will hear the regulator cycle and notice the sheath air pressure change to compensate for the liquid height change.

Figure 7.4-1 Troubleshooting Fluidics for No Sheath Flow



1. Move the jumper on the Pneumatic Interface card from E1- E2 to E2- E3.
2. Connect your DVM to TP11. Connect (tee) your pressure gauge to the sheath regulator output.
3. At the Cytometer Control screen, set **Sheath Pressure** to 12 psi.
4. DVM should read 8.00 V.
   a. If not, do steps 1 through 3 of Pneumatic Interface Card Adjustment under Heading 4.5.
   b. If you cannot obtain the indicated voltages, the circuit card is defective or the card is not receiving all the necessary power supply voltages. Check J10 for +15, -15, +24, +5 V.
   c. If you still cannot obtain the correct reading, the Pneumatic Interface card is defective or the card is not being correctly set by the CPU. Replace the card or inspect/replace the ribbon cable to Sensor I/O card.
5. If you obtained the correct reading in step 4, the regulator is being set for 12 psi.
6. Measure the air pressure at the regulator output. If it is 12 psi, the pressure is acceptable. If the pressure is not correct, adjust the regulator:
   a. Adjust the Range pot on the regulator until you read 12 psi.
   b. At the Cytometer Control screen, set **Sheath Pressure** to 6 psi. The voltage at TP11 should change to 4.00 V.

      If not, there is an electronic problem that must be corrected before proceeding. See step 4.
   c. Adjust the Zero pot so the gauge reads 6 psi.
   d. Repeat steps a through c until the pressure is correct at both levels. If the correct pressures cannot be achieved and the correct pressure (30 psi) is available to the regulator, the regulator is defective.
7. Once the regulator is adjusted correctly, place your DVM lead on TP4. Read the liquid pressure sensor output. The voltage here depends on the sheath liquid pressure.

   Note: You will read about 10 V at 15 psi and 8 V at 12 psi, or:

   $$\text{Volts} = \left(\frac{\text{sheath liquid pressure}}{15}\right) \times 10\text{Volts}$$

   The sheath liquid pressure is the same as the sheath air pressure only if there is no liquid in the system or the sheath tank level is the same as the sensor level (this is true when the bottle is half full). Since 1 psi = 27.68 in. of water and the liquid level in the bottle differs by 7 inches from empty to full, the liquid pressure at the sensor will be 0.25 psi higher when the bottle is full than when it is empty.
8. Fill the sheath bottle halfway with sheath fluid, connect your meter toTP4 and adjust the voltages:
   a. Set **Sheath Pressure** to 12 psi. You should read 8.00 V on your meter. If not adjust R75 for the correct voltage.
   b. Set **Sheath Pressure** to 6 psi. Wait for the pressures to stabilize then verify that you meter reads 4.00 V. If not, adjust R76 as needed.
   c. Repeat steps a and b until you read the correct voltage for both pressures.

Note: If the correct voltages cannot be obtained in this step, the in-line sheath pressure sensor or the Pneumatic Interface card may be defective.

9. If the voltage is correct but the display on the Cytometer screen is not, the problem is the Sensor Interface card.
10. Return E2-E3 to E1-E2 and readjust pneumatics as needed using Pneumatic Interface Card Adjustment.

Sample Pressure Problems

This system is simple to troubleshoot if you remember that all the Pneumatic Interface card does is send a voltage to the regulator which represents the desired pressure. The regulator does all the work; it contains a pressure sensor and controls to the pressure to match the requested pressure. The sample pressure sensor on the card is only used to obtain a pressure value to display on the Cytometer screen.

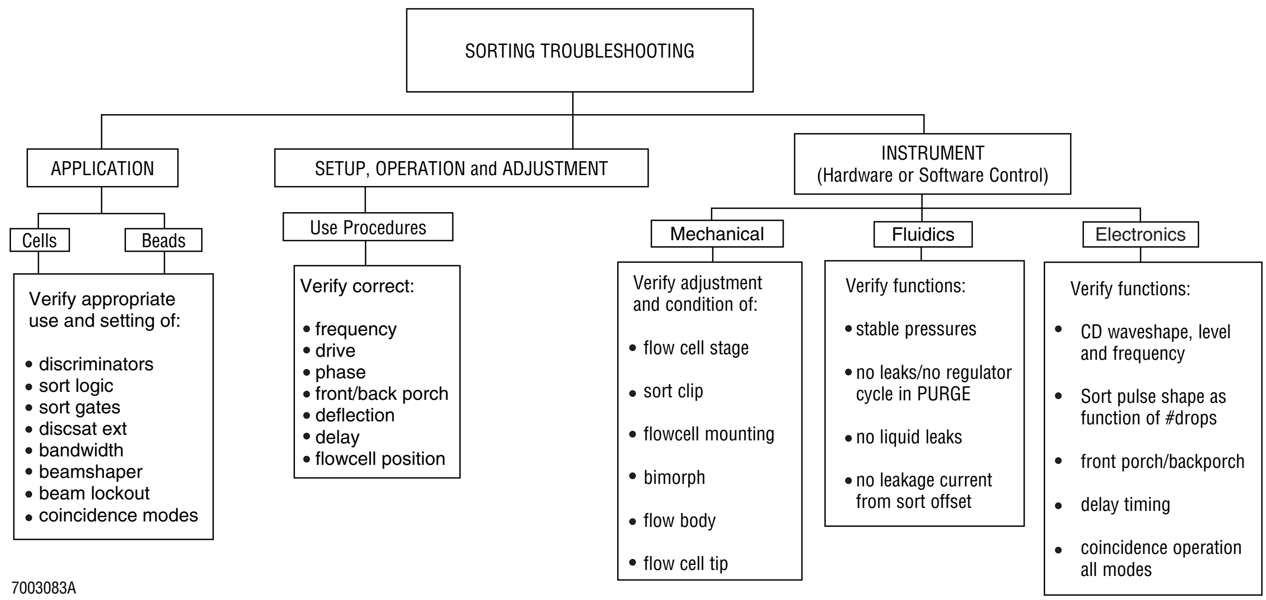
1. Connect your pressure gauge at the sample regulator output.
2. Connect your DVM to TP8.
3. Set **Sample Pressure** to 15 psi.
4. You should read 10 V on your meter. If not, do steps 1 through 4 of Low-Bleed Regulator Adjustment Procedure for Sample Regulator under Heading 4.5, PNEUMATICS SYSTEM.
5. If you cannot obtain the correct reading in step 4, the Pneumatic Interface card is defective or the card is not being correctly set by the CPU. Replace the card or inspect/replace the ribbon cable to Sensor I/O Card.
6. If you obtained the correct reading in step 4, the regulator is being set for 15 psi.
7. Measure the air pressure at the regulator output.
   - If it is 15 psi, the regulator is correctly set.
   - If the pressure is not correct, calibrate according to Pneumatic Interface Card Adjustment under Heading 4.5 procedure. If the correct pressure cannot be achieved and correct pressure is available to the regulator, then the regulator is defective.
8. Once sample pressure is present, you can check the sample pressure display circuit:
   a. Measure voltage at TP2. Voltage should be 8 V ±0.01 V.
   b. If voltage at TP2 is not correct, adjust R51 as needed. If 8 V cannot be achieved, the card is defective.
   c. Measure voltage at TP7. Voltage should be proportional to sample pressure of 8 V ±0.01 V. If there is no output, the sensor on the card is defective.

7.5 TROUBLESHOOTING THE SORT SUBSYSTEM

Purpose

Do this procedure to troubleshoot sort problems. Use Figure 7.5-1 and Table 7.5-1 as guides.

Figure 7.5-1 Troubleshooting Sort Problems



Tools/Supplies Needed

❑ DVM.

Sort Current Leakage Test

1. Put system in Sheath.
2. Ensure Sort Test, Sort Right and Left Enable are off.
3. Use your DVM to measure voltage on the sample insertion rod of the flow cell body.
4. Adjust Stream Offset and Deflection percent for maximum voltage. You should read at least 80 V.
5. Reduce Deflection percent to 0.0
6. Remove the alligator clip from sample intro rod and connect to one of your meter leads. Connect the other meter lead to the sample intro rod.
7. Set your meter to measure mA.
8. Slowly increase the deflection percent while watching the meter's display.
   - If more than 1 mA of current is detected, there is an unwanted current path from the sheath liquid to ground. The problem can be a cracked fitting or a metal bulkhead fitting (all tubing carrying fluids that connect to the flow cell must go through plastic feed-thru fittings).

Table 7.5-1 Troubleshooting Sort Problems

Condition	Symptom	Problem	Corrective Action
No droplet breakoff	Adjusted crystal drive; frequency did not respond	HV Deflection plates arced, causing crystal drive circuit to lockup	1. Turn off Cytometer (save settings first, if desired). 2. Clean and dry the deflection plate and power system on.
		Blown fuse on Sort Output card.	Replace the fuse.
		Power supply problem	Check the power supply.
Mist in sort area	Mist appears in sort area. Wet plates. Liquid buildup on top of sort tubes. Poor sort purity.	Satellite drops downstream have not recombined.	1. Adjust crystal drive frequency while looking at drops downstream to ensure that no satellite droplets (Figure 7.5-2) are present. 2. Dry the plates.
Fanning between side and center streams.	Constant fan of side streams into center stream. Wet plates. Liquid buildup on top of sort tubes. Poor sort purity and recovery.	Filament connecting last attached drop is too thick or too thin.	Adjust crystal drive setting to tighten the side streams.
		Data rate is too high for particular sample type.	1. Lower the data rate. 2. Filter the sample with nylon mesh. 3. Dry the plates.
		Incorrect phase setting.	Change the phase setting.
	Irregular fanning of side streams into center stream. Valves (regulators) sound like they are constantly cycling (clicking).	Sheath pressure is not constant.	1. Find leak in sheath and fix. 2. Replace the regulator.
	Fanning recurs shortly after “clear” or “debubble” temporarily fixes the problem.	Pinched sheath tubing.	Follow sheath tubing from the flow body to release the constriction. **Note:** Tugging gently on the sheath tubing at the point before it disappears behind the sort collection area may alleviate the problem.

Table 7.5-1 Troubleshooting Sort Problems *(Continued)*

Condition	Symptom	Problem	Corrective Action
Fanning between side and center streams *continued*.	There is a blackish/greenish buildup on the insertion rod in the flow body.	Insertion rod is corroded causing an irregular droplet charge and flow cell plugs.	Fix this problem before continuing the sort.
		Flow-through fittings on the valve bracket assembly are wrong (metal), or one of the correct flow-through fittings (PVC) is cracked.	Find cracked or damaged fitting and clean entire system of the metal fillings created from the electrolysis of the rod as instructed under Heading 4.11, SORT SENSE FLOW CELL ADJUSTMENT AND VERIFICATION.
	Fanning occurs after replacement or adjustment of flow cell.	Loose flow body. Loose flow cell tip.	Ensure the flow cell and the flow body are sufficiently tightened.
		Loose alligator clip.	Oil the hinge on the alligator clip.
		Bimorph is unstable.	Replace the bimorph using an enclosed bimorph.
Breakoff point is unstable.	Last attached drop slowly moves up and down. Three minutes after the last attached drop looks good, it varies again.	Leak in pneumatics system.	Find leak and repair.
		Old sample tubing.	Replace sample tubing with new.
		Crack or slight clog in flow cell.	Inspect flow cell and replace, if necessary.
		Bimorph is unstable.	Replace the bimorph using an enclosed bimorph.
	Breakoff is stable for 1 to 2 hours then never returns to the same place until the next morning.	Room temperature is too high. **Note:** A room temperature change of >1°C in 1 hour can cause a change in droplet formation.	Check the daily temperature variation and ensure the operating temperature is 18-29°C (64-84°F). Adjust air conditioning as needed.
Sample run affects breakoff point.	When RUN is pressed and sample "push" is over, the breakoff point moves down one drop.	Air in the sample line. Disturbance in the sample flow as it enters the sheath.	1. Remove the sample and press **CLEAR** several times to get the last droplet back to the reference cursor. 2. Ensure the side streams are stable and there is no wisping. 3. Without a sample on, press **RUN** to backflush the sample line and to clear out the remaining air bubbles. While still in RUN, put the sample on. The sample is automatically run; the push occurs for the specified time frame and the last attached drop should not move.

Table 7.5-1 Troubleshooting Sort Problems *(Continued)*

Condition	Symptom	Problem	Corrective Action
Low frequency required to establish breakoff	Frequency is normally around 30 kHz, but now gives high breakoff below 20 kHz.	Pinched sheath tubing.	Follow sheath tubing from the flow body to release the constriction. **Note:** Tugging gently on the sheath tubing at the point before it disappears behind the sort collection area may alleviate the problem.
		Clogged sheath filter.	Change the sheath filter.
		Sheath pressure too low.	Increase sheath pressure to 12 psi.
		Large orifice (140 µm) flow cell tip installed.	Verify that the correct tip is installed.
Low crystal drive required to establish breakoff.	Crystal drive % is usually 60-75%; now only 10% or less is needed to obtain a high droplet breakoff point.	Small orifice (76 µm) flow cell tip installed.	Verify that the correct tip is installed.
		Clog in flow cell.	Remove and clean the flow cell. Inspect before replacing.
Sort streams waver.	Sort streams waver from side to side with all three streams in sync.	Sort collection area door is open, or there is no filter in the biohazard filter slot behind the sort collection area.	Close the sort door, and ensure that there is a biohazard filter in the slot.
	Sort stream deflection oscillates; sometimes it is too narrow, other times it is okay.	Wet deflection plates.	Remove the plates. Clean with deionized water. Dry thoroughly and replace.
		Bubble in the flow cell.	Press **CLEAR** several times until wavering stops.
		Sample tubing needs to be changed.	Change the sample tubing.
No deflection.	No deflection of side streams when Sort Test is on and deflection is approximately 85%.	Deflection plates arced and shorted out.	1. Press **RESET** on the Cytometer. If that does not work, power down the system then power up again. 2. Clean and dry plates before replacing.
		Blown fuse on Sort Output card.	Replace the fuse.

Table 7.5-1 Troubleshooting Sort Problems *(Continued)*

Condition	Symptom	Problem	Corrective Action
Low sort purity.	Run of sorted samples is <90%.	Coincidence and/or PPU not used in sort setup.	Enable Coincidence Abort and PPU.
		Sort tubes too close to center stream.	Increase deflection 5-10%, and move sort tubes out slightly.
		Sort regions include unwanted cells not seen on histogram being displayed.	Include all histogram gates in the sort equation.
		Discriminator set too high.	Lower the discriminator setting.
		Sort delay and phase not set properly.	Review sort setup procedure and ensure the correct drop delay is sorted.
		Too many drops sorted.	Use a 1-drop sort window.
		Sort tubes too low	Use the sort tube holders to raise the 12 x 75 mm tubes up to the correct height within the sort area.
		Plastic tubes were used for sorting.	Use glass tubes with 200-500 µL of media in the bottom.
		Breakoff is unstable.	Check stability of LAD and retain stable system.
Low sort recovery.	Less than 50% of the sort count is in the sort collection tube.	Sort tubes are not catching all of the sort stream.	1. Use the sort tube holders to raise the 12 x 75 mm tubes up to the correct height within the sort area. 2. Increase deflection. 3. Use glass tubes with 200-500 µL of media in the bottom.
		Too few drops sorted.	Use a 3-drop sort window.
		Sort delay or phase not set properly.	Review sort setup procedure and ensure the correct drop delay is sorted.

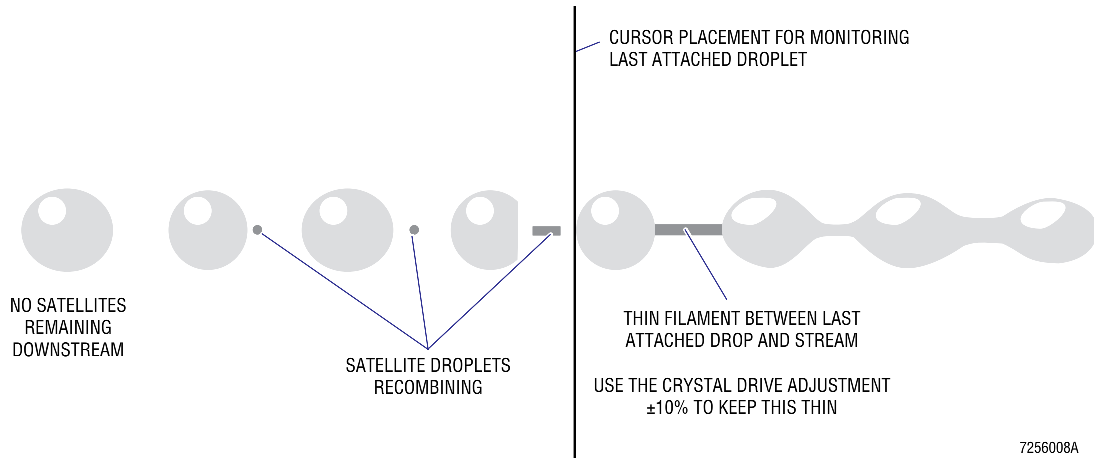
Table 7.5-1 Troubleshooting Sort Problems *(Continued)*

Condition	Symptom	Problem	Corrective Action
Last attached drop moves more than 3 drops when focusing the drops.	The last attached drop moves more than 3 drops downstream (to the left) when the frequency is adjusted for drop clarity.	The correct frequency has not been determined.	Find the point that gives the second highest breakoff and continue with step 4 under Drive Frequency Sort Settings of Heading 4.2, SORT WAVEFORM VERIFICATION AND ADJUSTMENT PROCEDURE.
		The bimorph has not sufficiently warmed up.	Allow the bimorph to warm up at least 30 minutes before determining the correct crystal frequency.
	A particular frequency gives the highest breakoff, but when the frequency is adjusted 0.1% up or down, the last attached drop moves downstream and becomes very unfocused.	There is a clog or bubble in the flow cell tip and/or body.	Press **CLEAR** three times, then press **DEBUBBLE**. Clean the flow cell.
Insufficient droplet breakoff with 90% crystal drive.	When crystal drive is 90% or greater, the droplet breakoff is still too far downstream; which means it is still below the ground plate when all the way down.	Hydraulic or pneumatic leak or problem.	1. Check the sheath tank cap, sheath filter, sheath tubing through the system. Use soapy water to find the leaks when bubbles occur. 2. Check that both the sheath and sample regulators are working properly. Rapid occurrence of the regulator cycling (<5 to 10 seconds between repetitive cycling) indicates a problem.
		Flow cell or body is not tightened properly.	Verify that the flow cell and body are properly tightened.
		Flow cell is clogged or cracked.	Remove and clean flow cell. Inspect before replacing. If cracked, replace.
		There is a bubble in the flow cell.	Press **CLEAR** three times, then press **DEBUBBLE**.
Double side streams.	There is a second side stream between the correct stream and the center on the other side.	Front Porch is not set correctly.	With Sort Test on and 3-drops sorted, adjust the Front Porch while watching the side streams until they form a single side stream. **Note:** Default setting is 8.
		Flow cell is suspect.	Replace the flow cell.

Table 7.5-1 Troubleshooting Sort Problems *(Continued)*

Condition	Symptom	Problem	Corrective Action
Cannot get tight side streams.	There is no phase setting that produces a clean side stream.	The stage housing for the enclosed bimorph may not be secured tight enough, which causes a slight vibration in the bimorph assembly.	Remove the Newport label on the bimorph stage and tighten the two screws that preload the bearings in the stage housing. Do this carefully to remove any free movement and to avoid binding movement of the housing.
Wide or fanning center stream.	Center stream is fuzzy; not a clean, single stream.	Back porch is not set correctly.	With Sort Test on and 3-drops sorted, adjust the Back Porch while watching the center stream until it forms a single side stream. Default setting is 2.
Cannot get sharp, focused drops downstream.	There is no frequency setting that produces sufficiently focused and clean drops.	Hydraulic or pneumatics leak or problem.	Check the sheath tank cap, sheath filter, sheath tubing through the system. Use soapy water to find the leaks when bubbles occur.
	Satellite drops never disappear.	Bubble or clog in flow cell.	Press **CLEAR** three times, then press **DEBUBBLE**.
Drops are unstable after a period of time.	Drops not stable for more than 3 or 4 hours.	Instabilities with fluidics.	1. Change the sheath pressure slightly up or down (±1.0 to 2.0 psi). 2. Complete sort setup beginning with Drive Frequency Sort Settingsunder Heading 4.2.
	More than a 10-15% change in the crystal drive is needed to achieve the droplet breakoff attained earlier that day.	Bimorph is unstable.	Replace the bimorph and verify it is tightly secured.

Figure 7.5-2 Satellite Droplets



7.6 RESETTING CYTOMETER SOFTWARE LOCKUPS

ATTENTION: These reset techniques only apply to those Cytometers containing an External Memory card. The older 256K Memory card does not respond in the same manner.

Purpose

Reset the CPU's memory anytime the Cytometer is locked up (not responding properly). If, after resetting the Cytometer CPU, the Cytometer is still locked up, clear the CPU's memory to restore normal operation.

Tools/Supplies Needed

- ❑ None.

Reset the Cytometer CPU

1. If the CPU locks up, locate the RESET switch on the switch panel.
2. Press and release the RESET switch to reset the Cytometer CPU.
3. If proper functioning is restored, resume normal operation.

   Note: If the Cytometer is still locked up, the CPU's memory must be cleared to restore normal operation. Follow the instructions under heading Clear the CPU's Memory (on the Cytometer) as written.

Clear the CPU's Memory (on the Cytometer)

Note: Clearing the CPU's memory allows an operator to easily reprogram the memory on the Cytometer without having to wipe out the memory from the Service screen.

1. Locate the RESET switch on the switch panel.
2. Press and hold the RESET switch for approximately 10 seconds until a tone is heard from the External Memory card. (The External Memory card is located in the Multibus card cage.)
3. At the Workstation keyboard, press F9.
4. When the prompt to transfer the program into the Cytometer appears, select **Yes** to initiate memory transfer from the Workstation to the CPU. The number "1" appears on the Cytometer right monitor and begins to sequence as the files are transferred.
5. Wait while the number displayed on the right monitor sequences (or counts) from 1 to approximately 425. This process takes several minutes.
6. Resume normal operation.

ILLUSTRATIONS

TABLES

8.1 MASTER PARTS LIST IN NUMERICAL ORDER BY CATEGORY

The parts are listed alphabetically by category and in order by part number. See Table 8.1-1 through 8.1-23.

Table 8.1-1 Autoclone Sorting Option

Part Number	Description	Figure	Item
1007908	Pin, threaded	8.2-24	6
1018230	Bellows	8.2-24	5
1018721	Cover, tray	8.2-23	11
1020199	Tray	8.2-23	12
1022047	Holder, cylinder	8.2-24	1
1022048	Shaft, Autoclone Sorting Option beak	8.2-24	2
1022049	Plate, Autoclone Sorting Option beak	8.2-24	3
2508022	Coupling, shaft	8.2-23	5
3501018	Motor, stepper, 1.8 degree angle, 0.44 shaft length	8.2-23	4
3505035	Motor, stepper, 1.8 degree angle, 5.5 Vdc	8.2-23	13
4703037	Resistor, 4.99 kOhm		
4703052	Resistor, 10 kOhm		
4818006	Switch, opto, 0.200 gap	8.2-23	9
4836701	Switch, opto, 0.1 gap	8.2-23	7
5103013	Switch, snap-action	8.2-23	6
5415377	Socket, modified	8.2-26	2
6028266	Cable, flex circuit, 14 in.	8.2-23	2, 3
6028268	Cable, main, Autoclone Sorting Option	8.2-25	1
6232037	Cylinder, air, single-acting	8.2-24	7
6704311	Deflection body	8.2-19 8.2-26	7 3
6704313	Autoclone Sorting Option mechanism	8.2-25	3
6705191	Card, Autoclone Sorting Option	8.2-25	2
6705328	Card, Interconnect	8.2-23	1
6705329	Card, Position Detection	8.2-23	8
6856635	Bezel, cover	8.2-26	1
6858187	Tray support sub-assembly	8.2-23	10
6858880	Beak, Autoclone Sorting Option	8.2-24	4
6912869	Kit, Autoclone Sorting Option.	8.2-23	

Table 8.1-2 Beam Shapers

Part Number	Description	Figure	Item
6856826	Standard, 6.5 µm ht., 151 µm width, 8 mm front focal length, 80 mm rear focal length, (sub-assembly for package) PN 6604660	8.2-20	4
6858427	Optional, 8 µm ht., 151 µm width, 10 mm front focal length, 80 mm rear focal length, PN 6604895		
6856696	Standard, 12 µm ht., 51 µm width, 15 mm front focal length, 60 mm rear focal length, PN 6604657	8.2-19	5
6857111	Optional, 12 µm ht., 70 µm width, 15 mm front focal length, 80 mm rear focal length, PN 6604656	8.2-19 8.2-20	5 4
6858019	Standard, 12 µm ht., 151 µm width, 15 mm front focal length, 80 mm rear focal length, PN 6604658	8.2-20	4
6857269	Standard, 6 µm ht., 112 µm width, 15 mm front focal length, 80 mm rear focal length, PN 6604659		
6857630	Standard, 17 µm ht., 34 µm width, 40 mm front focal length, 80 mm rear focal length		

Table 8.1-3 Cables

Part Number	Description	Figure	Part Number
3814178	Light cable, 0.125 in. diam., 60 in. long		
3814181	Light cable, 0.094 in. diam., 72 in. long		
6027510	Printer, parallel		
6027693	Printer, serial, MDAD System		
6027803	Bullet-bullet coax cable, 27 in.		
6027937	Data transfer, serial 9 pin, Cytologic		
6027939	Data transfer, serial 25-pin, Cytologic		
6027967	Cable assembly, Cytometer, 7.5 in. long		
6027992	KIR 3 communication, 41 in. long	8.2-7	4
6028002	Cable assembly, Cytometer, ground, 5 in. long	8.2-14	4
6028009	Data lister out, external		
6028016	Laser control cable, W49	8.2-35	6
6028018	Charge cable, sort transistor, W30	8.2-10	4
6028020	Pneumatics, sensor motor cable, sensor interface	8.2-10	3
6028021	Serial communications cable	8.2-7 8.2-10	14 17
6028022	60 POS, bitmap memory, ribbon	8.2-10	8, 11
6028023	Scope signal cable	8.2-10	9, 12
6028024	Pneumatic, CPU	8.2-10	13

Table 8.1-3 Cables *(Continued)*

Part Number	Description	Figure	Part Number
6028025	Data lister out, internal	8.2-7 8.2-10	13 7, 18
6028026	Data taker, PMT interface, DAC		
6028038	Data acquisition cable	8.2-10	10, 14
6028040	±36 V cable, W19	8.2-10	5
6028045	Dual CRT, W35	8.2-7	3
6028067	Serial communication, 9-25 pin		
6028070	BNC cable assembly, W31	8.2-7 8.2-10	19 16
6028086	Cable assembly, fluid detector	8.2-18	15
6028086	Fluid detector, SN4 cable, behind sample station	8.2-18	15
6028092	Pneumatic, PCB to sheath transducer cable, W45		
6028122	Bimorph cable		
6028144	Camera, video cable, ground mixer		
6028210	Back flow linkage	8.2-10	1
6028211	Serial, 9-25 pin, IBM adapter, 1 ft. long		
6028218	Cable assembly with interlock, Argon laser	8.2-34	5
6028256	Interlock cable, HeCd laser	8.2-38	4
6028266	Flex circuit cable, Autoclone Sorting Option, 14 in.	8.2-23	2, 3
6028268	Cable, main Autoclone Sorting Option	8.2-25	1
6028278	SCSI host, ribbon cable		
6028302	Power module control, 25-pin, external		
6028304	Cytometer 12		
6028371	Fiber optics, glass		
6028374	Analyzer card, W52	8.2-10	2
6028884	Data lister out, EMC		
6856953	Harness, W12	8.2-10	6
6858355	Connector panel, Elite Analyzer	8.2-10	15

Table 8.1-4 Cards

Part Number	Description	Figure	Item
2016127	PC - Bus, monochrome, 25 in. long		
2016378	Memory Expansion, 2 MB, 32 bit	8.2-28	7
2016413	Video, Elite, VGA SW setting		
2016424	Controller, Intel disk, floppy	8.2-28	6
2016526	Computer, 486-33, ISA, 8 MB RAM		
2016545	1522A Adapter, SCSI Host, Tahiti IIM		
2306042	Easy Artist, crystal oscillator, clock		
6702608	Sort Transistor	8.2-9 8.2-12	22 17
6702664	Serial I/O Card	8.2-9 8.2-11	2 3
6703761	DT Interface	8.2-9 8.2-11	3 4
6703772	Digiscope	8.2-9 8.2-11	6 8
6703941	PMT DAC R card		
6704008	PMT Gated Amp	8.2-8 8.2-9	6 33
6704009	Scat/Aux Gated Amp	8.2-8 8.2-9	11 28
6704089	Dual CRT Controller	8.2-9 8.2-11	5 7
6704139	ADC and PSH Control Card	8.2-12	9
6704142	Data Lister Out	8.2-8 8.2-9 8.2-12	23 15 10
6704147	Interface and Scaler	8.2-8 8.2-9 8.2-12	20 17 13
6704164	Mux and Scope Interface	8.2-8 8.2-9 8.2-12	27 11, 23 6
6704169	Quad Peak Sense and Hold (PSH) #1	8.2-8 8.2-9 8.2-12	26 13 8

Table 8.1-4 Cards *(Continued)*

Part Number	Description	Figure	Item
6704169	Quad Peak Sense and Hold (PSH) #2	8.2-8	25
		8.2-9	12
		8.2-12	7
6704185	Prism and Sort Window	8.2-8	22
		8.2-9	16
		8.2-12	11
6704186	Bitmap and Sort Decision	8.2-8	21
		8.2-12	12
6704188	Sort Oscillator	8.2-9	20
		8.2-12	15
6704198	Sort Delay	8.2-9	19
		8.2-12	14
6704201	Sort Output	8.2-8	17
		8.2-9	21
		8.2-12	16
6704241	Quad 7 Microsecond Delay #1	8.2-8	9
6704241	Quad 7 Microsecond Delay #2	8.2-8	8
6704327	±36 V Regulator	8.2-13	1
6704441	KIR 3	8.2-7	2
6704463	Dual Laser Control	8.2-9	7
		8.2-11	9
6704552	Pneumatic Interface Card Used only with Norgren pressure regulators	8.2-4	3
6704660	Camera Interface	8.2-9	8
		8.2-11	6
6704665	Lister AT A & B		
6704679	Motor Controller	8.2-13	5
6704697	PMT Gated Amp R		
6704798	Pin Hole Driver		
6704893	Gated Amp Control	8.2-8	2
6704904	Quad 20/40/60 Microsecond Delay #1	8.2-8	10
		8.2-9	29
6704904	Quad 20/40/60 Microsecond Delay #2	8.2-8	7
6704906	Pulse Generator and Clock	8.2-12	1
6704907	Pulse Generator and Clock	8.2-8	30
		8.2-9	9
6704913	Extender, Data Acquisition/Gated Amp power	8.2-8	15

Table 8.1-4 Cards *(Continued)*

Part Number	Description	Figure	Item
6704914	Extender, Motor Controller power		
6704915	Extende,r Multibus power		
6704916	Extender HV DAC		
6704933	Cytometer Computer Backplane	8.2-11	1
6704959	Pulse Pileup Detector/TOF	8.2-9	10
6705073	Sample Tube Indicator		
6705117	Sensor Interface	8.2-8	16
6705182	Peak Scatter Sensor	8.2-19	9
6705191	Autoclone Sorting Option	8.2-25	2
6705246	3 PMT Sub SW-R	8.2-8	4
		8.2-9	27, 34, 35
6705249	Dual FL SW-R	8.2-8	3
		8.2-9	36
6705252	Scat/CV SW-R	8.2-8	14
		8.2-9	25
6705327	Gated Amp Control R3	8.2-9	38
6705328	Interconnect card, Autoclone Sorting Option	8.2-23	1
6705329	Position Detection, Autoclone Sorting Option	8.2-23	8
6705369	Peak Scatter/Mux SW-R	8.2-8	13
		8.2-9	26
6705400	Peak ADC PSH Control card	8.2-8	24
		8.2-9	14
6705504	Pneumatic Interface R2, used with low bleed regulators, PN 6232490 and 6232491.		
6705681	Analyzer Interface	8.2-9	18
6705694	Sort Oscillator R2	8.2-8	18
6705954	Pulse Pileup Detector/TOF2	8.2-8	28
		8.2-12	5
6706034	Sort Delay R 3	8.2-8	19
6706232	Bitmap and Sort Decision R2	8.2-8	21
		8.2-12	12
6706571	Cytometer CPU card with PROMs Computer IBC-86C	8.2-11	2
6804637	Bertan Supply No. 5 card (PMT HV Bertan)		
7000681	External Memory	8.2-9	4
		8.2-11	5

Table 8.1-5 Covers/Doors

Part Number	Description	Figure	Item
1017262	Cover, plate bottom, HeCd laser	8.2-33	5
1018721	Cover, tray, Autoclone Sorting Option	8.2-23	11
1018859	Cover, pinch valve	8.2-17	4
6856509	Door, bottle tray	8.2-3	1
6856889	Door, pneumatic, side	8.2-4	5
6858097	Door, analyzer sample stage		

Table 8.1-6 Cytometer

Part Number	Description	Figure	Item
1010932	Nut, upper, insertion rod gland	8.2-31 8.2-32	2 2
1018859	Cover, pinch valve, sample drawer, Analyzer	8.2-17	4
1018860	Template, FALS detector, sheet		
1019167	Switch, overlay and membrane	8.2-1	16
1019224	Probe, pick-up	8.2-17	5
1019224	Tube, sample, sample drawer	8.2-17	5
1019958	Bar, obscuration, jet-in-air	8.2-16	3
1020069	Tube holders, 12 x 75	8.2-1	22
1020377	Gusset, LH, rear	8.2-37	1
1020909	Switch, membrane, Elite Analyzer		
1020913	Block, sample station, sample drawer	8.2-17	12
1022054	Shield, splash, sample drawer	8.2-17	1
2105057	Power connector	8.2-14	5
2305005	Crystal, bimorph		
2306042	Clock, crystal oscillator		
2306046	Transducer, sheath pressure	8.2-6	8
2510014	Mount, shock	8.2-14	3
2603012	Fan/blower, rear door fans, 79 CFM, 115 Vac	8.2-4	4
2603065	Fan blower, 112CFM, 115Vac	8.2-14 8.2-15	2 1
2802006	Screw, obscuration bar	8.2-16	1
2806201	Screw, RPL, sample drawer, 6-32 x 0.38	8.2-17	8
2807038	Setscrew, jet-in-air ring, 6-32 x 0.250	8.2-16	4
2839058	Screw, self-locking, sample drawer, 4-40 x 0.18	8.2-17	6

Table 8.1-6 Cytometer *(Continued)*

Part Number	Description	Figure	Item
2851876	Thumbscrew, RPL, sample drawer, 6-32 x 0.25	8.2-17	7
3814172	Stage, flow cell	8.2-19 8.2-20	2 14
3814173	Stage, beam	8.2-19 8.2-20	3 16
3814223	Mirror, 45D, adaptor	8.2-29 8.2-36	3 6
3814271	Positioner, ultra-compact, flow cell stage	8.2-31 8.2-32	1 1
4506029	Relay, K1 and K2, 2 pole, 120 Vac	8.2-27	2
4508007	Relay, K3 and K4, 100 - 140 Vac, 3 - 32 Vdc input	8.2-27	2
5120109	Switch, drip chamber	8.2-5	3
5120178	Switch, pressure, 25 psi	8.2-5	6
5120196	Switch, rotary, dual-CRT	8.2-7	10
5120199	Switch, waste filter		
5609034	Transformer, T3, 100/220/240	8.2-27	4
5609035	Transformer, T1 and T2, 100/220/240	8.2-27	5
6027967	Cable assembly, ground, 7.50 in.	8.2-14	6
6028002	Cable assembly, ground, 14 ga., 5.0 in.	8.2-14	4
6211015	Valve, actuator, pilot, silver	8.2-18 8.2-19	14, 23 1
6214106	Valve, check, for 0.125 in. tubing	8.2-6	11
6214108	Valve, check		
6232205	Fitting, barbed, hose, sample drawer, elbow, 0.125 i.d. to 10-32 nylon, white		
6703950	Backplane, HV DAC/PMT	8.2-13	3
6704171	Backplane, data acquisition card cage	8.2-12	18
6704753	Camera stage	8.2-1	1
6704754	Deflection body	8.2-19 8.2-21	7 1
6704787	Sensor, scatter, non-peak		
6705402	Sensor, scatter, peak, R2	8.2-19	9
6705493	Power supply, 3000V	8.2-15	2
6705597	Sensor, scatter, analyzer	8.2-19 8.2-20	9 8
6705987	Deflection plates, jet-in-air flow cell tips		

Table 8.1-6 Cytometer *(Continued)*

Part Number	Description	Figure	Item
6854718	Sensor, fluid SN4, behind sample station		
6856241	Bottle, flow cell cleaning		
6856505	PMT #4	8.2-1 8.2-22	13 1
6856675	Holder, deflection body		
6856677	Compressor, module 115V		
6856678	Trap, water (air/water separator)	8.2-3	8
6856696	Beamshaper, 15 x 60 mm.	8.2-19	5
6856703	CRT, dual-assembly, with covers	8.2-1 8.2-7	12 1
6856762	Flow body, thin fixed rod (old mount)	8.2-31 8.2-32	3 3
6856826	Beamshaper, 8 x 60 mm.	8.2-20	4
6856833	PMT #1, #2, #3, and #5	8.2-1 8.2-22	14 2, 3, 4, 5
6856842	Drawer, sample collection	8.2-1	3
6856866	Door, rear, transformer rack	8.2-14	1
6856930	Table top		
6856967	Vial cap		
6856970	Motor, mixer	8.2-1	5
6856971	Sample station	8.2-1	4
6856984	Bubble chamber, lower		
6856985	Bubble chamber, upper	8.2-6	1
6857012	Shield, CRT housing		
6857048	Holder, base and end cap, sample tube		
6857066	Snout, pinhole	8.2-20	10
6857133	Shutter assembly	8.2-19 8.2-20	4 2
6857170	Blower, laser box, assembly		
6857276	Light source, fiber optics, PN 3814177		
6857369	Bottle, rinse		
6857388	Valve, pinch, double-action	8.2-19	1
6857400	Holder, prism	8.2-19	10
6857598	Ring, jet-in-air	8.2-16	2
6857636	Adapter, interlock bypass	8.2-16	5
6857637	Bracket, interlock relay, subassembly	8.2-16	6

Table 8.1-6 Cytometer *(Continued)*

Part Number	Description	Figure	Item
6858907	Door, sample drawer, Analyzer	8.2-17	13
6858908	Drain, sample, sample drawer	8.2-17	10
6858923	Cap, sample tube	8.2-17	2

Table 8.1-7 Filters

Part Number	Description	Figure	Item
1015831	Biohazard filter, container		
3802050	515 nm LP/laser blocker filter,		
3802073	530 SP filter		
3814044	2.0 ND filter,		
3814069	635 nm BP		
3814222	beam splitter, 633 nm		
3814227	395 nm BP filter, air-cooled laser		
6232143	Hydrophobic, round-disk, 0.3 micron filter	8.2-5	10
6232473	Sheath, 0.2 micron	8.2-3	18
6232489	Waste, 0.2 micron	8.2-3	14
6857204	Holder, filter		
6857205	675 nm BP filter, 3814139		
6857206	575 nm BP filter with holder, Elite ESP, 3814135		
6857207	600 nm DL filter, Elite ESP, 3814138		
6857208	550 nm DL filter, Elite ESP, 3814067		
6857209	525 nm BP filter, Elite ESP, 3814134		
6857210	488 nm BK filter, Elite ESP, 3802072		
6857211	488 nm BP filter, Elite ESP, 3814137		
6857212	488 nm DL filter, Elite ESP, 3814136		
6857370	Mirror filter, 3805018		
6857500	1.0 ND filter and 530 SP assembly, 3802048, 3802073		
6858219	381 nm BP filter, HeCd laser, 3814264		
6858220	440 nm DL, 3814199		
6858249	633 nm LP, 3814191, 3814266		
6858371	1.0 ND filter, Elite ESP, 3802048		
6859060	610 nm BP filter, Elite ESP, 3814273		
6859061	640 nm DL, Elite ESP, assembly, 3814137		

Table 8.1-8 Flow Cell Area

Part Number	Description	Figure	Item
1019009	Flow cell holder, Elite Analyzer Bracket, holder, flow cell mount	8.2-20	5
1019539	Bracket, interlock, Elite Analyzer	8.2-20	1
1010933	Clamp, nut, Elite Analyzer	8.2-20	7
1016487	Tubing, sample, 7.0 in. Elite Analyzer	8.2-20	12
1020903	Bracket, tube holder, Elite Analyzer	8.2-20	15
2851558	Screw, machine, 25-20 x 1.0 Elite Analyzer	8.2-20	3, 11
3210004	Tubing silicone, sample, 0.010 i.d., Elite Analyzer	8.2-17 8.2-19 8.2-20	3 11 6
3814172	Stage, flow cell, Elite Analyzer	8.2-19 8.2-20	2 14
3814173	Stage, beam, Elite Analyzer	8.2-19 8.2-20	3 16
6211015	Valve, actuator, pilot	8.2-5 8.2-18 8.2-19	8 14, 23 1
6602642	Flow cell, Biosense	8.2-32	5
6704311	Deflection body, Autoclone Sorting Option	8.2-19 8.2-26	7 3
6704754	Deflection body, standard	8.2-19 8.2-21	7 1
6705182	Sensor, peak scatter, R	8.2-19	9
6705402	Sensor, scatter, R2	8.2-19	9
6705597	Sensor, scatter, Elite Analyzer	8.2-19 8.2-20	9 8
6856101	Flow cell tip, 100 micron, with thin flow body		
6856191	Flow cell tip, Elite Analyzer	8.2-20	9
6856511	Flow cell tip, 76 micron, Sort Sense		
6856696	Beam shaper, 15x60 mm	8.2-19	5
6856826	Beam shaper, 8 x 80 mm, Elite Analyzer	8.2-20	4
6857066	Pinhole assembly, Elite Analyzer	8.2-20	10
6857111	Beam shaper, 15 x 80 mm, Elite Analyzer	8.2-20	4
6857133	Shutter, single-acting, Elite Analyzer	8.2-19 8.2-20	4 2
6857388	Valve, pinch, double-acting	8.2-19	1
6857400	Prism holder	8.2-19	10

Table 8.1-8 Flow Cell Area *(Continued)*

Part Number	Description	Figure	Item
6858019	Beam shaper, 15 x 80 mm	8.2-20	4
6858222	Deflection body holder, 3,000 V, old	8.2-19	6
6859007	Deflection body holder, new	8.2-19	6
6859300	Flow cell tip 100 µ 3x	8.2-31	5
6859313	Flow cell tip 76 µ 3x	8.2-31	5
6859314	Flow cell tip Profile 3x		
6859315	Pinhole Snout 3x	8.2-19	8
6859397	Flow cell tip, ESP Sort Sense, 140µ	8.2-31	5

Table 8.1-9 Fuses

Part Number	Description	Figure	Item
5102002	250 , 0.13A, NB fuse		
5102007	250 V, 3A		
5102013	250 , 3A, slo blo		
5102021	250 V, 2A, slo blo		
5102026	250 V, 0.25A, slo blo		

Table 8.1-10 Hardware

Part Number	Description	Figure	Item
1005697	Fitting, barbed, metal, 0.062 i.d.	8.2-6	7
1007908	Pin, threaded, Autoclone Sorting Option	8.2-24	6
1010932	Nut, upper, insertion rod gland	8.2-31 8.2-32	3 2
1010933	Clamp, nut, Elite Analyzer	8.2-20	7
1015427	Fitting, restrictor tubing bottom, 0.020 i.d.		
1016827	Plate support, upright rear, HeCd laser	8.2-33	7
1017261	Plate, front, mounting, HeCd laser	8.2-33	8
1017262	Cover, plate bottom, HeCd laser	8.2-33	5
1017375	Plate, rear mounting, HeCd laser	8.2-33	4, 6
1017501	Mount, pinch valve, analyzer	8.2-18	11
1017916	Base	8.2-17	9
1018879	Plunger, test tube	8.2-18	3
1019539	Bracket, interlock, Elite Analyzer	8.2-20	1

Table 8.1-10 Hardware *(Continued)*

Part Number	Description	Figure	Item
1019658	Bracket, air duct, HeNe/Argon Laser	8.2-35	2
1020271	Brackets, mounting, Argon laser	8.2-34	6, 7
1020382	Bracket	8.2-3	
	- Waste		12
	- Sheath		16
1020901	Holder, pinch valve, analyzer	8.2-18	21
1020903	Bracket, tube holder, Elite Analyzer	8.2-20	15
1022190	Screw, 0.25 - 0.28 x0.94, HeCd Laser 74	8.2-33	2
2306051	Bracket, mounting, transducer	8.2-6	9
2508022	Coupling, shaft, 0.188 and 0.250 i.d. ends, Autoclone Sorting Option	8.2-23	5
2515062	Spring, analyzer, 0.344 o.d. x 0.62 x 0.25	8.2-18	4
2523062	O-ring sea, 0.187 i.d. x 0.050I	8.2-18	18
2523549	Spring, 0.250 o.d., HeNe/Argon laser	8.2-35	3
2523656	Washer, 0.25 bolt, 0.63 o.d., HeCd Laser 74	8.2-33	3
2751587	Nut, hex, 25-20 UNC, Argon laser		
2851598	Nut, hex, 37-16, Argon laser	8.2-34	2
2851762	Screws, machine, metric, M6x25 mm, Argon laser	8.2-34	3, 9
2806201	Screw, 6-32 x 0.38, analyzer sample	8.2-17	8
2814004	Screw, 25-20 x 0.50, HeNe/Argon laser	8.2-35	14
2821010	Nut, self-locking, analyzer	8.2-18	19
2822033	Nut, hex, 47-32 UNS x 0.562A, Analyzer	8.2-18	2
2826051	Washer, split-lock, 0.26 i.d. x 0.49 o.d., Argon laser	8.2-34	4
2830014	Grommet, sample tube, 0.375 i.d. x 0.625 o.d. x 0.250	8.2-1	20
2839039	Screw, self-locking, 6-32 x 0.37, analyzer	8.2-18	20, 24
2839043	Screw, self-locking, 6-32 x 0.62, analyzer	8.2-18	16
2839058	Screw, self-locking, 4-40 x 0.18, analyzer	8.2-17	6
2851558	Screw, machine, 25-20 x 0.62, Elite Analyzer	8.2-20	3, 11
2851836	Screw, thumb, 6-32 x 0.25, analyzer	8.2-20	13
2851876	Thumbscrew, RPL, analyzer sample	8.2-17	7
3814206	Screw, metric, micro-optical rail		
6011002	Tie, wire, nylon, 0.14 x 6.7	8.2-35	7
6211015	Valve, actuator, analyzer, 7/16 bore	8.2-5	8
		8.2-18	14, 23
		8.2-19	1
6216012	Spacer, cylinder, 0.500 i.d. x 0.562 o.d. x 0.062	8.2-18	13
6216129	Fitting, hose barb, T, brass, 0.187 i.d.	8.2-6	10

Table 8.1-10 Hardware *(Continued)*

Part Number	Description	Figure	Item
6216345	Gasket, #10, black	8.2-18	10
6232086	Fitting hose barb, 0.062 i.d. to 10-32 thread	8.2-18	17
6232092	Fitting, quick-connect, white female	8.2-3 8.2-4	2 8
6232093	Fitting, quick-connect, white male		
6232104	Fitting, hose barb, T, 0.115 to 0.180 i.d.	8.2-6	5
6232124	Fitting, hose miniature, 10-32, analyzer	8.2-18	9
6232205	Fitting, barb, elbow, nylon, white, 0.125 i.d. to 10-32	8.2-17 8.2-18	11 7
6232208	Fitting, hose barb, elbow, nylon, white, 0.093 i.d. to 10-32, flow cell stage	8.2-31 8.2-32	4 4
6232215	Fitting, brass T, 0.250 in. o.d.		
6232304	Fitting, quick-connect, panel mount, orange		
6232305	Fitting, quick-connect, insert, orange		
6232306	Fitting, quick-connect, insert, blue		
6232309	Fitting hose, quick-connect, panel mount, blue	8.2-3 8.2-4	17 7
6232417	Fitting, hose barb, 0.062 in. i.d., PVC	8.2-6	6
6232418	Fitting, hose barb, 0.115 - 0.180 in. i.d., PVC	8.2-6	4
6232449	Fitting, panel mount bottle, orange male		
6232450	Fitting, panel mount bottle, blue male	8.2-3 8.2-4	15 13
6232451	Fitting, panel mount bottle, white male	8.2-3 8.2-4	3 12
6232452	Fitting, panel mount bottle, yellow male	8.2-3	9
6232472 6232475 6232522	Fitting, quick-connect, 0.025 o.d., single-conn., white Fitting, quick-connect 0.125 flow, single-conn., white Fitting, quick-connect, 0.375 o.d., single-conn., white		
6803642	Valve, pinch, double-acting, Analyzer	8.2-18	1
6855763	Valve, pull-apart, standard pinch, analyzer	8.2-18	12
6856321	Bracket, wired, sort		
6857112	Feet, mounting, HeNe	8.2-35	5
6857133	Shutter, single-acting, Elite Analyzer	8.2-19 8.2-20	4 2
6858368	Bracket, flow cell, bimorph sub-assembly	8.2-31 8.2-32	6 6

Table 8.1-10 Hardware *(Continued)*

Part Number	Description	Figure	Item
6858369	Bracket, mount, Sensor card, analyzer	8.2-18	5
6858370	Bracket, mount, pinch valve, analyzer	8.2-18	22
6912841	Hardware, laser mounting, Innova, 90-5		
6912844	Hardware, laser mounting, Innova 305		
6912869	Autoclone Sorting Option Sorting Option		
6912870	Hardware, laser mounting, Cyonics Argon Laser		
6912871	Hardware, laser, mounting, HeNe, red, Uniphase		
6913205	Hardware, laser mounting, Enterprise, Innova,		
69132278	Hardware, laser mounting, Spectrum		

Table 8.1-11 Kits

Part Number	Description	Figure	Item
6912185	He-Cad Expander		
6912268	Filters, Biohazard replacement		
6912655	HeCd, Indo optics		
6912749	HeNe, red, Uniphase	8.2-36	1
6912750	Argon laser, Cyonics	8.2-36	2
6912792	Innova 305	8.2-36	3
6912795	Piezo flow cell mount		
6912797	PMT 5 upgrade, before SN U07122		
6912798	Gated Amp upgrade, after SN U07122		
6912801	HeCd laser		
6912827	Gated Amp upgrade, before SN U07122		
6912831	Innova 305 water cooled laser		
6912832	90-5 water cooled laser		
6912833	Cover upgrade		
6912834	Optics, HeCd 74 laser	8.2-38	1
6912938	Filters, sheath and waste, 0.2 micron		
6912971	He-Cad, Omnichrome, mounting hardware		
6912985	Beam translator, with laser	8.2-35	9
6913056	PMT 5, switch amps		
6913251	Snout upgrade		
6913419	PMI, Service kit		
6913199	Enterprise laser, Coherent		

Table 8.1-11 Kits *(Continued)*

Part Number	Description	Figure	Item
6913206	Enterprise optics		
6913211	Spectrum laser, Coherent		
6913212	HeNe laser, Melles Griot Green		
6913229	HeCd 74, Omnichrome laser		
6913250	Sort enhancement, switch amp, after SN U07122		
6913251	Snout upgrade		
6913253	Sort enhancement, pre-switch amp, before SN U07122		
6914966	Software driver, HP 1600C (for International use only)		
6914967	HP 1600C Color Printer ink cartridges		
6914968	HP 1600C Color Printer		
6914969	Printer, color, Elite		
6915005	90 MHz Pentium, full workstation upgrade		
6915006	90 MHz Pentium upgrade, Elite		
6915082	Elite 256 Sort Upgrade		

Table 8.1-12 Lamps

Part Number	Description	Figure	Item
3908021	Lamp, indicator, 24V, amber		
3908022	Lamp, indicator, 24V, red		
3908023	Lamp, indicator, 24V, green		
3908025	Lamp, halogen, 21 V, 150 W	8.2-13	9

Table 8.1-13 Lasers

Part Number	Description	Figure	Item
3203037	Foam, strip, panel, laser interlock assembly		
3814232	Argon Ion laser	8.2-34	1
3814233	Table, top optical, Argon laser	8.2-34	8
3814244	Power supply, HeCd laser	8.2-38	1
3814286	Mirror, 325	8.2-36	4
6027225	Line cord, Omnichrome HeCd laser		
6028256	Cable, interlock, Omnichrome HeCd laser	8.2-38	4
6705218	Laser HeCd 74	8.2-33	1
6856681	Upright, mounting, Argon laser	8.2-35	12

Table 8.1-13 Lasers *(Continued)*

Part Number	Description	Figure	Item
6856859	Table top, laser mount	8.2-4	1
	Laser Alignment Targets With 3 Target Holes		
6857274	Large	8.2-36	9
6857236	Small	8.2-36	10
6857286	Cover, laser interlock assembly		
6857334	Interlock, laser, with red pin		
6857347	Duct, intake, laser	8.2-35	15
6857424	Duct plenum, HeNe laser	8.2-35	1
6857483	Interlock, defeat bar, red, L-shaped		
6857515	HeNe laser	8.2-35	4
6857516	Argon laser, tested	8.2-35	13
6857611	Stage, beam splitter, 488 nm	8.2-35	10
6857612	Mirror, HeNe laser	8.2-35	11
6857613	Stage, beam splitter, 325 nm		
6858243	Beam splitter, 633 nm light, HeNe laser		
6858244	Beam translator, UV light, either HeCd or Argon UV laser		
6858245	Beam translator, 488 nm light, Argon laser		
6858459	Target, multipositional, 3-hole, large	8.2-36	11
6912654	Kit, Laser, HeCd 74, Omnichrome		
6912749	Laser, red HeNe, Uniphase	8.2-36	1
6912750	Laser, air-cooled Argon, Cyonics	8.2-36	2
6912792	Laser, Innova 305, Coherent	8.2-36	3
6912834	Kit, HeCd 74 laser	8.2-38	1
6913199	Laser, Enterprise		
6913211	Laser, Spectrum		
6913212	Laser, He-Ne, green, Melles Griot		
6913229	Laser, Coherent		
6913248	Heat exchanger, Enterprise, 230 V		
6913249	Heat exchanger, Enterprise, 115 V		
7232344	Diode, Enterprise laser, starter		
7232500	Enterprise laser, laser head assembly		
7232501	Enterprise laser, power supply assembly		
7232502	Enterprise laser, heat exchanger, 115 Vac		
7232503	Enterprise laser, remote control		
7232504	Enterprise laser, remote defeat		
7232505	Enterprise laser, heat exchanger defeat		

Table 8.1-13 Lasers *(Continued)*

Part Number	Description	Figure	Item
7232506	Enterprise laser, power supply, air filter		
7232507	Enterprise laser, Temperature Sensor card		
7232508	Enterprise laser, hex wrench		
7232509	Enterprise laser, washer, hose,screen, fine		
7232510	Enterprise laser, starter module		
7232511	Enterprise laser, water flow transducer		
7232512	Enterprise laser, head pc card		
7232513	Enterprise laser, beam splitter assembly		
7232514	Enterprise laser, light pick-off, 488 nm		
7232515	Enterprise laser, light pick-off, UV		
7232516	Enterprise laser, cable, remote module		
7232517	Enterprise laser, cable, heat exchanger interlock, 25 feet		
7232519	Enterprise laser, cable, umbilical		
7232520	Enterprise laser, model 621, operator's manual		
7232521	Enterprise laser, cooling fan, power supply		
7232522	Enterprise laser, washer, hose, coarse screen		
7232523	Enterprise laser, power cord, 8 gauge		

Table 8.1-14 Miscellaneous

Part Number	Description	Figure	Item
2016298	Drive, floppy, 5.25 in.	8.2-28	4
2016328	Drive, floppy, 3.5 in.	8.2-28	5
2121023	Jumper, discrete wire, 2-position, single row		
2523451	O-ring, sheath tank cap, silicone		
2838068	Clamps, break-apart, metal		
2851763	Screw, machine, M6 x 12 mm		
2851920	Screw, machine, metric, M6 x 20 mm		
3814206	Rail, mounting, beam translator, micro-optical		
3814224	Rail, table, laser optical, 16.5 in.		
	Gloves, latex		
5415179	small/medium		
5415174	medium/large		
5415175	large/extra large		
6027225	Cord, 3-18 ga., HeCd laser	8.2-38	6

Table 8.1-14 Miscellaneous *(Continued)*

Part Number	Description	Figure	Item
6214106 6801536	Valves, check for 0.156 i.d. tubing, waste line	8.2-6	2, 11
6603488	DNA-Check Beads, kit		
6703953	Detector, pump motor, analyzer	8.2-18	6
6856479	Filter coax, ac		
6856911	Drawer, transformer	8.2-27	1
6857479	Computer box, 3100	8.2-28	1
6858680	Rack, Data Acquisition		
6912798	Amplifier, gated		
8547008	Fluid, sheath, IsoFlow, 3 liter		

Table 8.1-15 Optics

Part Number	Description	Figure	Item
3814233	Table, optical	8.2-34	8
6604656	Beam shaper, 15 x 80 mm, confocal, optional		
6604657	Beam shaper, 15 x 60 mm, confocal		
6604658	Beam shaper, 15 x 80 mm Δ 15		
6604659	Beam shaper, 15x 80 mm Δ 6		
6604660	Beam shaper, 8 x 80 mm Δ 15		
6604895	Beam shaper, 10 x 80 mm Δ 15, optional		
6856826	Beam shaper, 8 x 80 mm, Elite Analyzer	8.2-20	4
6857111	Beam shaper, 15 x 80 mm, Elite Analyzer	8.2-20	4
6857630	Beam shaper, 40 x 80, confocal		
6857634	Stage, beam splitter	8.2-29	5
6858019	Beam shaper, 15 x 80 mm Elite Analyzer	8.2-20	4

Table 8.1-16 Pneumatics

Part Number	Description	Figure	Item
1005697	Fitting, hose barb, metal, small, 0.062 i.d.	8.2-6	7
1020380	Bracket, rinse	8.2-4	10
2306046	Transducer, sheath pressure	8.2-6	8
2306051	Bracket, mounting, transducer	8.2-6	9
2603012	Blower, 79 CFM, 115 Vac	8.2-4	4

Table 8.1-16 Pneumatics *(Continued)*

Part Number	Description	Figure	Item
5120109	Switch, drip chamber	8.2-5	3
5120109	Switch, waste filter	8.2-5	4
5120109	Switch, system pressure	8.2-5	5
5120178	Manifold assembly, 25 psi	8.2-5	6
6028142	Cable assembly, sheath, service	8.2-3 8.2-4	6 9
6211015	Actuator, pilot, 7/16 bore	8.2-5 8.2-18 8.2-19	8 14, 23 1
6216129	Fitting, hose barb, T, brass, 0.187 i.d.	8.2-6	10
6232092	Fitting, quick-connect, white	8.2-3 8.2-4	2 8
6232096	Valve, solenoid	8.2-5	7
6232104	Fitting, hose barb, T, 0.115 to 0.180 i.d.	8.2-3 8.2-6	17 5
6232309	Fitting hose, quick-connect, panel mount, blue	8.2-3 8.2-4	17 7
6232413	Fitting, miniture, nipple, 10-32	8.2-5	8
6232417	Fitting, hose barb, 0.062 in. i.d., PVC	8.2-6	6
6232418	Fitting, hose barb, 0.115 - 0.180 in. i.d., PVC	8.2-6	4
6232449	Quick-connect, orange		
6232450	Fitting, panel mount bottle, blue male	8.2-3 8.2-4	9, 15 13
6232451	Fitting, panel mount bottle, white male	8.2-3 8.2-4	3 12
6232452	Fitting, panel mount bottle, yellow male	8.2-3	9
6232490	Regulator, sample (low bleed regulator)	8.2-5	1
6232491	Regulator, sheath (low bleed regulator) Used with Pneumatic Interface Card PN 6705504.	8.2-5	2
6704552	Pneumatic Interface Card Used only with Norgren pressure regulators	8.2-4	3
6801536	Valves, check for 0.156 i.d. tubing, waste line	8.2-6	2
6855931	Lines, air and vacuum		
6856539	Drawer, pneumatics		
6856634	Box, gauge and regulator, pneumatic housing		
6856677	Compressor, module 115V		
6856699	Pneumatics cabinet	8.2-4	N/A

Table 8.1-16 Pneumatics *(Continued)*

Part Number	Description	Figure	Item
6856859	Table top, laser mount	8.2-4	1
6856889	Door, pneumatic, side	8.2-4	5
6856984	Chamber, lower bubble	8.2-6	3
6856985	Bubble chamber, upper	8.2-6	1
6857576	Valve, pinch, double-acting	8.2-5	9
6857872	Rinse tank assembly	8.2-4	2

Table 8.1-17 Power Supplies

Part Number	Description	Figure	Item
2603058	Fan/blower, 90 CFM, 24 Vdc, 4.69 sq. in., 1 in. thick		
4004024	±90 Vdc, 0.2A, 115 Vac	8.2-13	14
4004074	Switcher, 400 W	8.2-13	8
4004084	Power conerter, 1-3 K Vdc out, 5-12 Vdc in		
6704960	Deflection	8.2-13	15
6705493	3000 V Deflection	8.2-15	2
6804637	PMT HV, Bertran	8.2-13	4
6853275	±15 V, 2.0 A	8.2-13	10
6854358	±15 V, 5.0 A	8.2-13	12
6856870	±15 V, 0.25 A, PMT	8.2-13	11
6856636	PMT HV	8.2-13	2
6857017	HeNe laser		
6857226	Fiber optics		
7260017	Computer, 220 W	8.2-28	2

Table 8.1-18 Printers

Part Number	Description	Figure	Item
2016675	HP Deskjet 1600C Color Printer		
2016691	Cartridge, ink, HP 1600C Color Printer, magenta		
2016692	Cartridge, ink, HP 1600C Color Printer, black		
2016693	Cartridge, ink, HP 1600C Color Printer, cyan		
2016694	Cartridge, ink, HP 1600C Color Printer, yellow		
4237252	Guide, installation, Elite color printer		
6027510	Cable, parallel printer		

Table 8.1-18 Printers *(Continued)*

Part Number	Description	Figure	Item
6417268	Driver, software, HP 1600C Color Printer		
6914968	Kit, HP 1600C Color Printer		
6914969	Kit, Elite color printer		

Table 8.1-19 Regulators

Part Number	Description	Figure	Item
6232490	Low Bleed Regulator (sample regulator) Used with Pneumatic Interface Card PN 6705504.	8.2-5	1
6232491	Low Bleed Regulator (sheath regulator) Used with Pneumatic Interface Card PN 6705504.	8.2-5	2

Table 8.1-20 Software

Part Number	Description	Figure	Item
6141379	Flownet, Profile, 3.5 in.		
6414357	Profile 2.26, 3.5 in.		
6414520	DOS 4.01, 5.25 in.		
6414852	OPTune®, 3.5 in.		
6414853	OPTune®, 5.25 in.		
6414976	Elite, version 4.00, disk #1		
6414977	Elite, version 4.00, disk #2		
6912816	Elite, version 4.0 with PrintQue		
6913114	Elite, version 4.01		
6915099	Elite, version 4.5		

Table 8.1-21 Tools

Part Number	Description	Figure	Item
4717730	Potentiometer, dual-CRT, trigger adjustable	8.2-7	9
7232508	Enterprise laser, hex wrench		

Table 8.1-22 Tubing

Part Number	Description	Figure	Item
1016430	Restrictor tubing, 2.0 in.		
1016487	Restrictor tubing, 7.0 in.	8.2-20	12
3202039	Waste-carrying tubing, 0.145 i.d.		
3203016	Pinch valve tubing, silicone, blue stripe, 0.079 i.d. x 0.031		
3210004	Sample line tubing, 0.010 i.d. x 0.036	8.2-17 8.2-19 8.2-20	3 11 6

Table 8.1-23 Workstation

Part Number	Description	Figure	Item
1018377	Pen, graphics pad		
1019318	Clip, AT		
1022173	Mouse pad		
2016161	Mouse, serial	8.2-2	1
2016279	Cartridge, disk removeable, Q-PAK		
2016280	Drive, 5.25 in., remoeable		
2016297	Disk controller, Profile XT		
2016298	Drive, floppy, 5.25 in., 1.6 MB	8.2-28	5
2016328	Drive, floppy, 3.5 in.	8.2-28	5
2016340	Disk controller, Profile AT, long board		
2016368	Cartridge, ribbon, Fujitsu printer		
2016381	Drive, floppy, 5.25 in., 1.2 MB		
2016383	Power supply, switching, 115 Vac/60 Hz, 230 Vac/120 Hz		
2016387	Workstation, Intel 301Z		
2016392	Keyboard, enhanced, 102-key		
2016413	Card, video, VGA SW		
2016416	Drive, non-removable, 85.32 MB	8.2-28	3
2016419	Card, Adapter, PC to Ethernet		
2016424	Card, Disk Controller, 1003 V	8.2-28	6
2016426	Drive, optical, 600 MB, 115 Vac		
2016427	Pack, Cartridge, optical disk		
2016447	Workstation, Intel 302		
2016457	Card, adapter, SCSI host		
2016458	Drive, disk, cartridge, SCSI, 20 MB		

Table 8.1-23 Workstation *(Continued)*

Part Number	Description	Figure	Item
2016468	Cartridge, data, removable disk, 21.4 MB		
2016500	Ribbon, printer, color, Star®		
2016501	Cartridge, ribbon, printer, black, Star NX-2480		
2016526	Card, Adapter, SCSI host		
2016527	Printer, dot matrix, Panasonic®		
2016545	Kit, adapter, SCSI host		
2016551	Cartridge, disk, removable, 90 MB		
2016556	Cartridge, printer, font, Microsoft®, HP Laserjet		
2016557	Printer, letter tray, Laserjet II P Plus		
2016558	Cartridge, printer, Deskjet 500		
2016560	Cartridge, toner, printer, Laserjet II P Plus		
2016568	Drive, disk, optical rewritable, 512, 650 MB		
2016569	Drive, disk, optical, 650 MB, write 1x-Worm 512		
2016570	Drive, disk, optical, 1 GB, write 1x-Worm 1024		
2016576	Cartridge, toner, printer, Laserjet IV		
2016577	Switch, data, manual, A/B		
2016592	Keyboard, 101-key, compact		
2016613	Computer, 486 DX2, 66 MHz, 16 MB RAM, ISA		
2016615	Cartridge, toner, printer, Laserjet 4P		
2016616	Printer, Laserjet 4P		
2016651	Monitor, 17 in., 1024x1024	8.2-2	5
2016652	Drive, disk, cleaning kit, 3.5 in.		
2016670	Computer, Pentium, 90 MHz, 16 MB RAM, 540 MB hard disk, drive	8.2-2	2
2016690	Card, Memory, PCMCIA, 512 K		
2121537	Connector, T, BNC, UG-274B		
2429044	Keyboard overlay, function strip, long		
2430458	Keyboard template, 2XL, label		
2906841	Terminator, BNC male, 50 Ohm, 0.5 W		
4004048	Battery, 3.0V, lithium		
4004070	Battery, 3.6V, NiCad		
6027510	Cable, printer, parallel		
6027937	Cable, data transfer, Cytologic, 9-pin		
6027939	Cable, data transfer, Cytologic, 25-pin		
6028009	Cable, Lister		
6028278	Cable, ribbon, SCSI, host		

Table 8.1-23 Workstation *(Continued)*

Part Number	Description	Figure	Item
6028302	Cable, power module control, 25-pin, external, 10 ft.		
6028304	Cable, Cytometer 12, 10 ft.		
6028371	Cable, fiber optic		
6414243	Disk, REM, flex, 5.25 in.		
6414379	Software, Flownet, 3.5 in.		
6414520	Disk, DOS 4.01, 5.25 in.		
6414535	Disk, Flownet, Node option		
6414852	Software, OPTune®, 3.5 in.		
6414853	Software, OPTune®, 5.25 in.		
6414976	Software, Elite 4.00, disk #1		
6414977	Software, Elite 4.00, disk #2		
6604245	Printer, Paintjet, Hewlett-Packard		
6604418	Card, MULTICYCLE®		
6703473	Card, Extender, Profile		
6703474	Card, extender driver, Profile		
6703672	Card, CRT contrast, Profile		
6704278	Card, motherboard, Faraday, Profile XT		
6704459	Card, Backplane, Profile AT		
6704473	Drive, extender, Flownet, 128K, Profile		
6704556	Interface, DT, Profile AT		
6704665	Card, Lister		
6704965	Interface, DT, Profile XT		
6705318	Transputer, cytometer, TSTD		
6705582	Card, Extender, XL		
6705815	Software library, XL		
6706418	Software library, Elite, version 4.02		
6855899	Bracket, trigger, Profile		
6856379	Lister 88, Profile		
6856736	Card, CPU, Profile AT		
6856752	CRT 2, Profile		
6857978	Drive, Panasonic, Profile XT		
6912111	Drive, 90 MB, Bernoulli, kit		
6912518	Switcher, replacement kit, Profile II		
6912579	Software, Easy 2.2		
6912612	Drive, 20 MB, Bernoulli, field-upgradable		

Table 8.1-23 Workstation *(Continued)*

Part Number	Description	Figure	Item
6912816	Software, Elite 4.0, with PrintQue		
6912866	FlowNet, network kit		
6912881	Cartridge, data, 21.4 MB		
6912970	Drive, optical, multi-function		
6912973	Switcher, 12V		
6913112	Drive, disk, 90 MB, Bernoulli, removable		
6913113	Cartridge, printer, Laserjet 4, with Font		
6913114	Software, Elite 4.01		
6913115	Printer, Laserjet 4P		
6913117	Printer, Deskjet 500, Hewlett-Packard®		
6913149	Labels, 100 sheets, 8.5 x 11 in.		
6913170	Labels, barcode generation kit		
6913258	Software, 1.5 XL, without cable		
6913259	Software, 1.5, with serial cable		
6913343	Labels, barcode, 3 rolls		
6913404	Drive, 150 MB, Bernoulli, ISA		
6913407	Drive, disk, Tahiti II, without SCSI card		
6915005	Kit, Pentium 90 MHz, full workstation upgrade		
6915006	Kit, Pentium 90 MHz upgrade		
6928258	Cable, serial communication, 9-25 pin		
7231244	Disk, service, pre-final test		
7260019	Battery pack, Intel		

8.2 ILLUSTRATED PARTS

Figure 8.2-1 Elite Cytometer, Right Panel Removed (See Table 8.2-1)

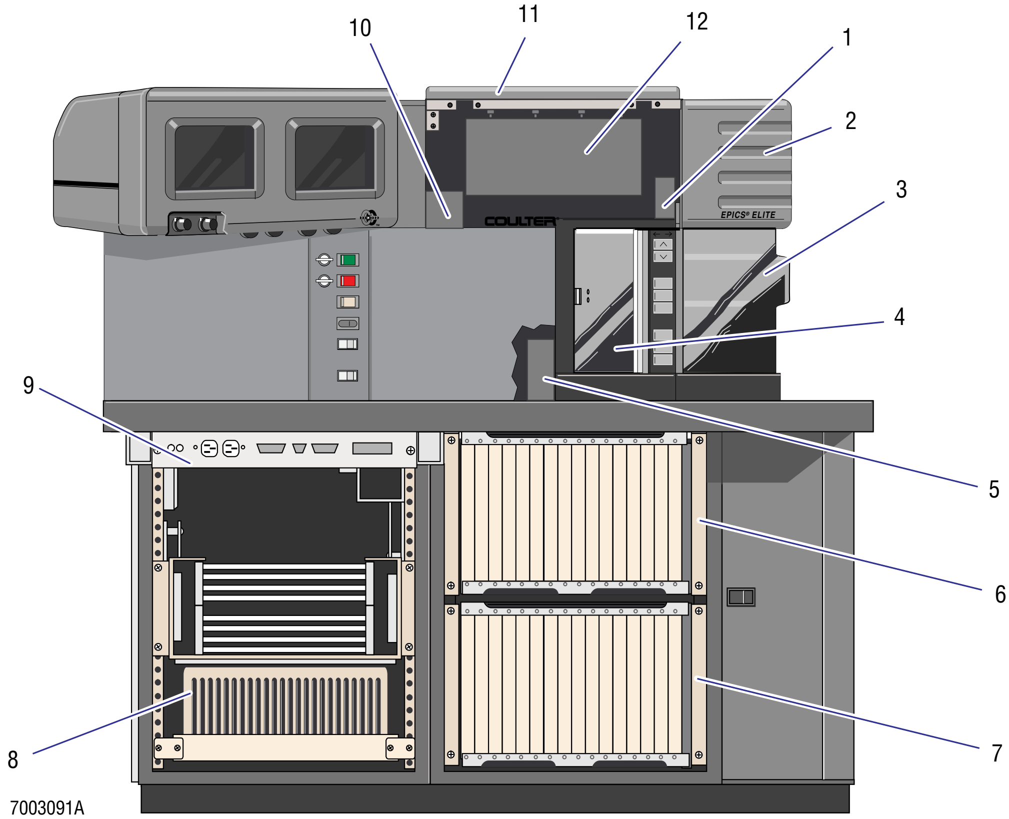


Table 8.2-1 Elite Cytometer, Right Panel Removed, Detail of Parts (See Figure 8.2-1)

Item	Part Number	Description	Item	Part Number	Description
1	6704753	Camera (inside)	7	N/A	Data Acquisition card cage
2	6602642	Flow cell (inside)	8	6856911	Transformer drawer
3	6856842	Sample collect drawer	9	6856950	Connector panel
4	6856971 6857203	Sample station Door	10	6856505	PMT 4 (inside)
5	6856970	Mixer motor assembly	11	6856833	PMT 5 (inside)
6	N/A	Gated Amp card cage	12	6856942	PMT plate (inside)

Figure 8.2-2 Workstation (See Table 8.2-2)

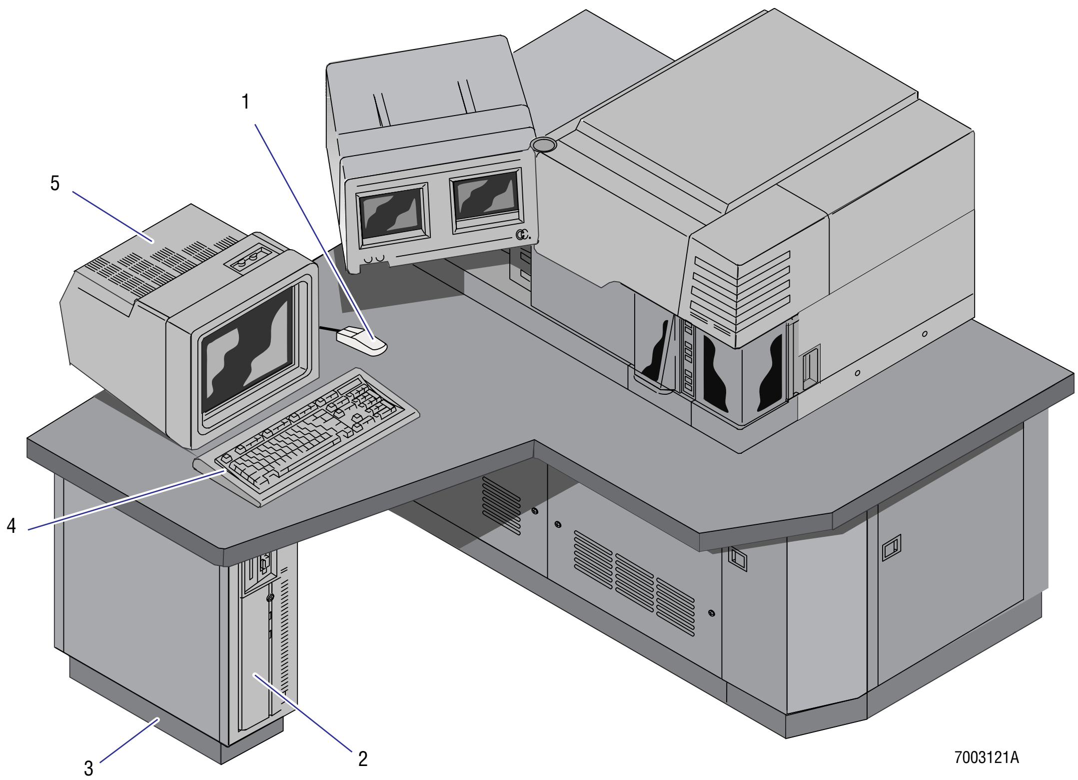


Table 8.2-2 Workstation, Detail of Parts (See Figure 8.2-2)

Item	Part Number	Description
1	2016161	Mouse
2	2016670	Computer, Pentium (Workstation pc)
3	6856936	PC Pedestal
4	2016592	Keyboard
5	2016651	Monitor, color, 17 in.
N/A	1411141	Chair
N/A	6914969	Printer, color

Figure 8.2-3 Sheath and Waste Compartment (See Table 8.2-3)

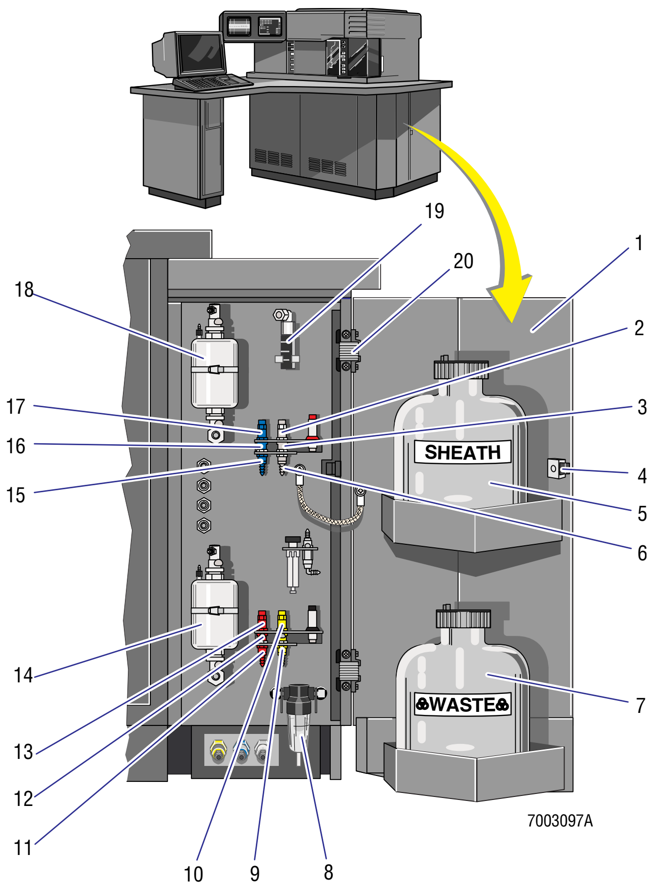


Table 8.2-3 Sheath and Waste Compartment, Detail of Parts (See Figure 8.2-3)

Item	Part Number	Description	Item	Part Number	Description
1	6856509	Bottle tray door	11	6232449	Quick-connect, connector, orange
2	6232092	Quick-connect, connector, white	12	1020382	Waste bracket
3	6232451	Quick-connect, body, white	13	6232304	Quick-connect, body, orange
4	2851668	Paddle latch	14	6232489	Gas filter, waste
5	6857374	Sheath bottle assembly	15	6232450	Quick-connect, connector, blue
6	6028142	Sheath service cable assembly	16	1020382	Sheath bracket
7	6857373	Waste bottle assembly	17	6232309	Quick-connect, body, blue
8	6856678 7260050 7260051	Water trap assembly Clear bowl assembly Filter element kit	18	6232473	Water filter, 0.2 micron
9	6232452	Quick-connect, connector, yellow	19	6216286	Muffler/breather
10	6232303	Quick-connect, body, yellow	20	2523515	Hidden hinge

Figure 8.2-4 Pneumatics Cabinet (See Table 8.2-4)

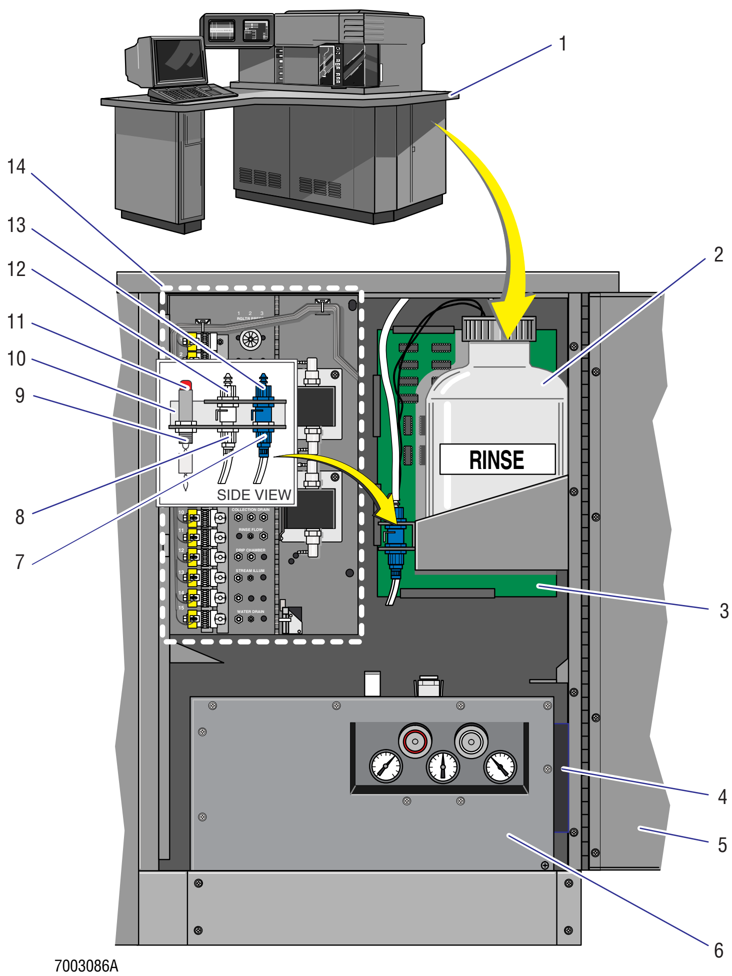


Table 8.2-4 Pneumatics Cabinet, Detail of Parts (See Figure 8.2-4)

Item	Part Number	Description	Item	Part Number	Description
1	6856859	Table, optical, laser mount	8	6232092	Quick-connect, body, white
2	6857872	Rinse tank assembly	9	6028142	Sheath service cable
3	6704552	Pneumatic Interface card	10	1020380	Rinse bracket
4	2603012	Fan/blower	11	6202814	Cable assembly
5	6856889	Pneumatics door	12	6232451	Quick-connect, connector, white
6	6856677	Compressor module	13	6232450	Quick-connect, connector, blue
7	6232309	Quick-connect, body, blue	14	6856539	Pneumatics drawer

Figure 8.2-5 Pneumatics Drawer (See Table 8.2-5)

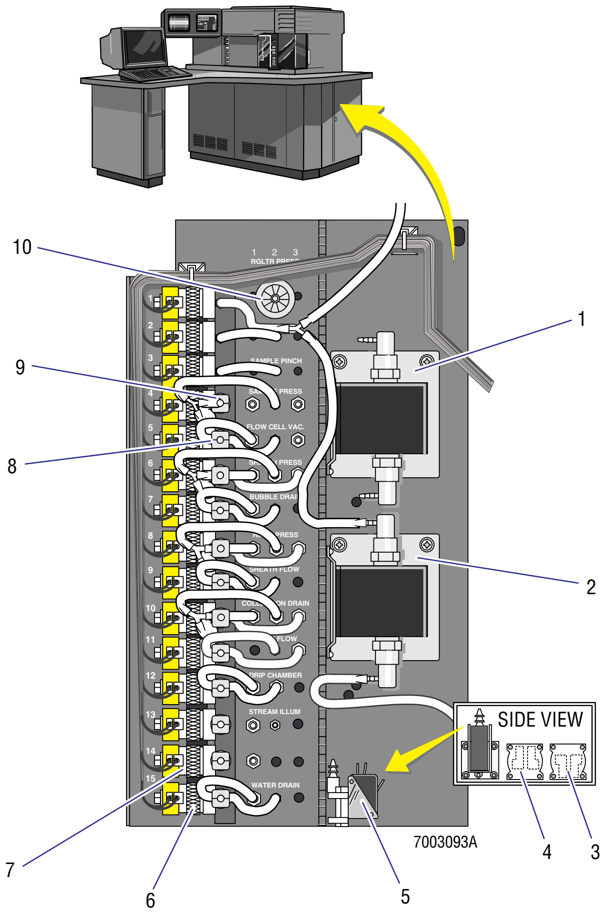


Table 8.2-5 Pneumatics Drawer, Detail of Parts (See Figure 8.2-5)

Item	Part Number	Description
1	6232490	Sample regulator
2	6232491	Sheath regulator
3	5120109	Drip chamber switch
4	5120109	Waste filter switch
5	5120109	Fluidics pressure switch
6	5120178	Pressure switch
7	6232096	Solenoid valve
8	6211015	Pilot actuator
	6232413	Nipple, 10-32
9	6857576	Double-acting pinch valve
10	6232143	0.3 micron filter

Figure 8.2-6 Pneumatics Panel, Rear View (See Table 8.2-6)

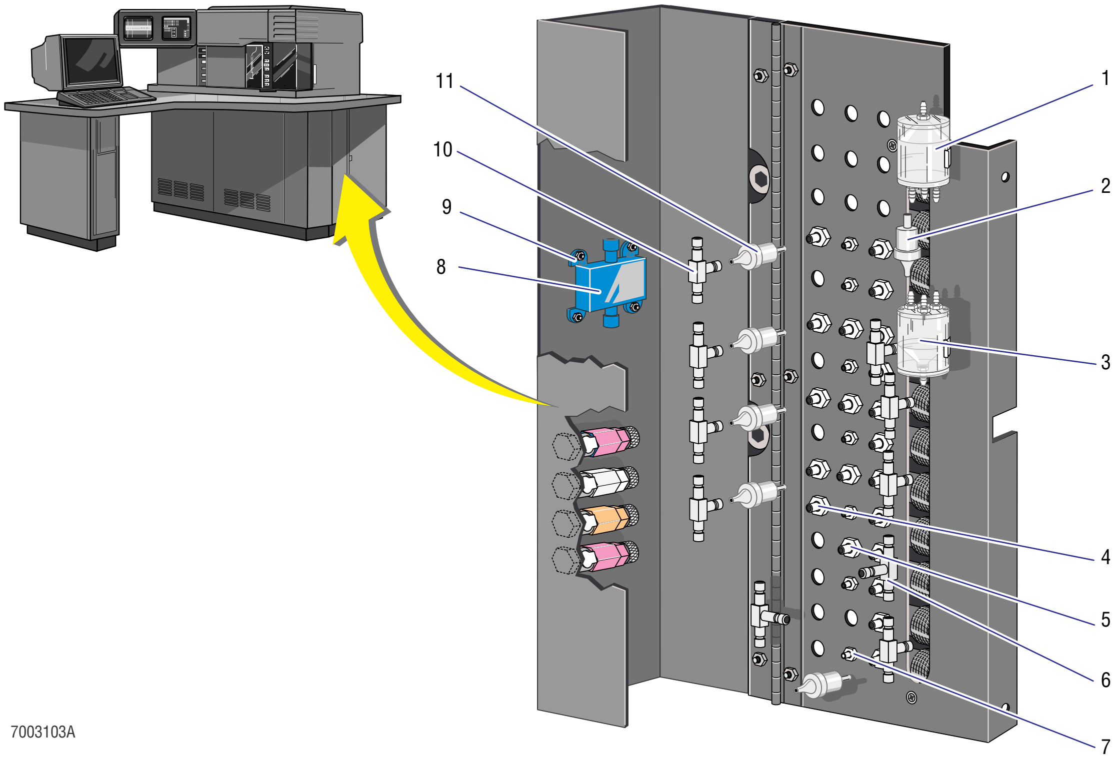


Table 8.2-6 Pneumatics Panel, Rear View, Detail of Parts (See Figure 8.2-6)

Item	Part Number	Description
1	6856985	Upper bubble chamber
2	6801536	Check valve #1
3	6856984	Lower bubble chamber
4	6232418	Large PVC barbed fitting
5	6232104	Large metal barbed fitting
6	6232417	Fitting, hose barb, 0.062 i.d., PVC
7	1005697	Small metal barbed fitting
8	2306046	Sheath pressure transducer
9	2306051	Transducer mounting bracket
10	6216129	T-fitting, brass, 0.187 i.d.
11	6214106	Check valve for tubing, 0.156 i.d.

Figure 8.2-7 Dual CRT and Connector Panel (See Table 8.2-7

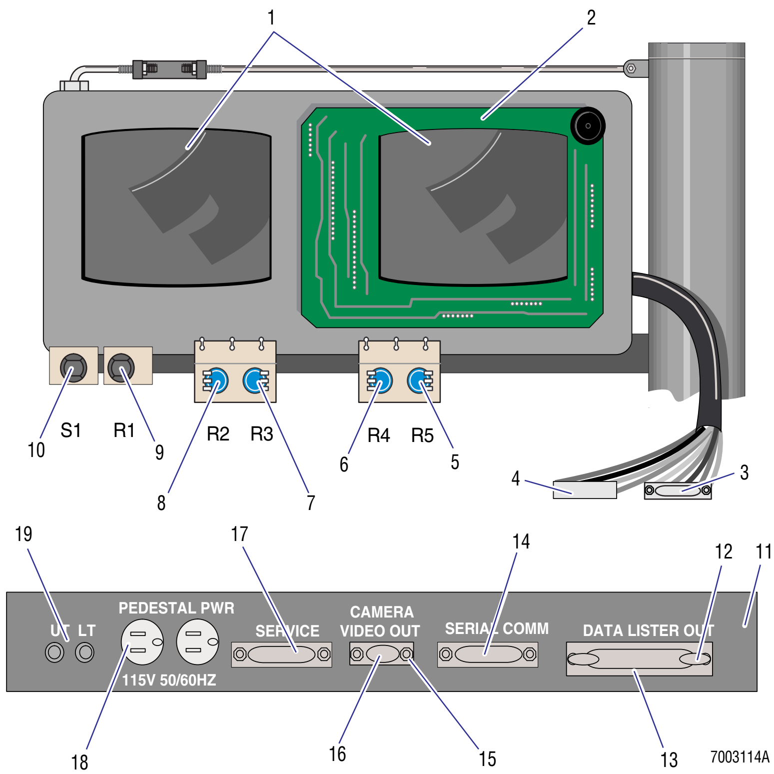


Table 8.2-7 Dual CRT and Connector Panel, Detail of Parts (See Figure 8.2-6)

Item	Part Number	Description	Item	Part Number	Description
1	6856703	CRT assembly, complete CRT assembly, without covers	11	6856950	Connector panel, front
2	6704441	KIR 3 card	12	2121586	Bail lock, D-type
3	6028045	Dual CRT cable	13	6028025	Data Lister Out (INT) cable
4	6027992	KIR 3 communication cable	14	6028021	Serial communication cable
5	4717714	Contrast control knob	15	2104261	Screw lock, female
6	4717714	Brightness control knob	16	6028113	AUX video (INT) cable
7	4717714	Contrast control knob	17	6028068	Service (INT) cable
8	4717714	Brightness control knob	18	2105017	Duplex outlet
9	4717730	Dual CRT potentiometer, trigger adjustable	19	6028070	BNC (W31) cable
10	5120196 1010165	Scope trigger, switch Scope trigger, knob			

Figure 8.2-8 Card Configuration, Elite (See Table 8.2-8

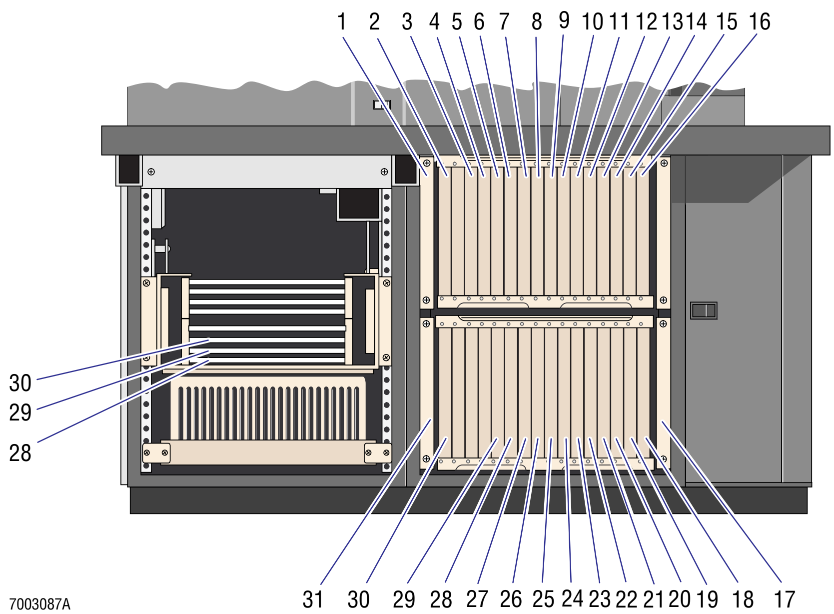


Table 8.2-8 Elite Card Configuration, Detail of Parts (See Figure 8.2-8)

Item	Part Number	Description	Item	Part Number	Description
1	N/A	Gated Amp card cage	16	6705117	Sensor Interface card
2	6704893	Gated Amp Control R2 card	17	6704201	Sort Output R card
3	6705249	Dual FL Amp SW-R card	18	6705694	Sort Oscillator R2 card
4	6705246	3 PMT Sub Amp SW-R2 card	19	6706034	Sort Delay R3 card
5	6705246	3 PMT Sub Amp SW-R1 card	20	6704147	Interface and Scaler card
6	6704008	PMT Gated Amp card	21	6704186 or 6706232	Bitmap and Sort Decision card Bitmap and Sort Decision R2 card
7	6704904	Quad 20/40/60 Microsecond Delay 2 card	22	6704185	Prism and Sort Window Test card
8	6704241	Quad 7 Microsecond Delay 2 card	23	6704142	Data Lister Out card
9	6704241	Quad 7 Microsecond Delay 1 card	24	6705400	Peak ADC and PSH Control card
10	6704904	Quad 20/40/60 Microsecond Delay 1 card	25	6704169	Quad PSH 2 card
11	6704009	Scat/Aux Gated Amp card	26	6704169	Quad PSH 1 card
12	6705246	3 PMT Sub Amp SW-R 2 card	27	6704164	Mux and Scope Interface card
13	6705369	Peak Scatter/Mux SW-R card	28	6705954	Pulse Pileup Det./TOF card
14	6705252	Scat/CV SW-R1 card	29	6784913	Gated Amp card (data acquisition)
15	6704913	Gated Amp Extender card (data acquisition)	30	6704907	Pulse Generator and Clock card

Figure 8.2-9 Card Configuration, Elite ESP (See Table 8.2-9

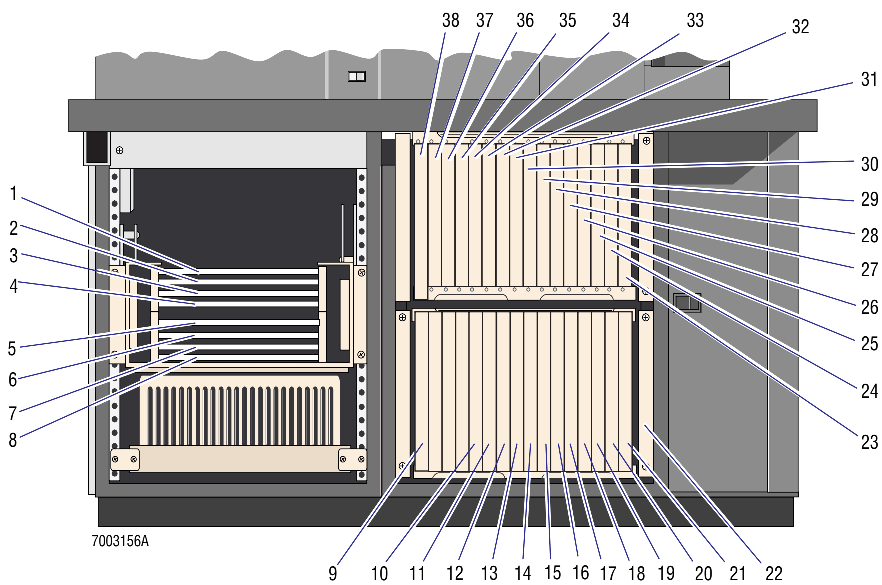


Table 8.2-9 Elite ESP Card Configuration, Detail of Parts (See Figure 8.2-9)

Item	Part Number	Description	Item	Part Number	Description
1	2016414	Intel/CPU 8086 card	20	6704188	Sort Oscillator card
2	6702664	Serial I/O card	21	6704201	Sort Output card
3	6703761	Data Taker Interface card	22	6702608	Sort Transistor card
4	7000681	External Memory card	23	6704555	Sensor Interface card
5	6704089	Dual CRT Controller card	24	6704164	Mux and Scope Interface card
6	6703772	Digiscope card	25	6705252	Scat/CV SW-R1 card
7	6704463	Dual Laser Control card	26	6705369	Peak Scatter/ Mux SW card
8	6704660	Camera Interface card	27	6705246	3 PMT Sub SW 1 card
9	6704907	Pulse Generator and Clock card	28	6704009	Scat/Aux Gated Amp card
10	6704959	Pulse Pileup Det./TOF card	29	6704904	Quad 20/40/60 Microsecond Delay 1 card
11	6704164	Mux and Scope Interface card	30	6704241	Quad 7 Microsecond Delay 1 card
12	6704169	Quad PSH 2 card	31	6704241	Quad 7 Microsecond Delay 2 card
13	6704169	Quad PSH 1 card	32	6704904	Quad 20/40/60 Microsecond Delay 2 card
14	6705400	Peak ADC PSH Control card	33	6704008	PMT Gated Amp card
15	6704142	Data Lister Out card	34	6705246	3 PMT Sub SW 1 card
16	6704185	Prism and Sort Window card	35	6705246	3 PMT Sub SW 2 card
17	6704147	Interface and Scaler card	36	6705249	Dual FL SW-R card
18	6705681	Analyzer Interface card	37	N/A	(Spare slot)
19	6704198	Sort Delay card	38	6705327	Gated Amp Control R3 card

Figure 8.2-10 Cable Interconnections, Elite and Elite Analyzer (See Table 8.2-10)

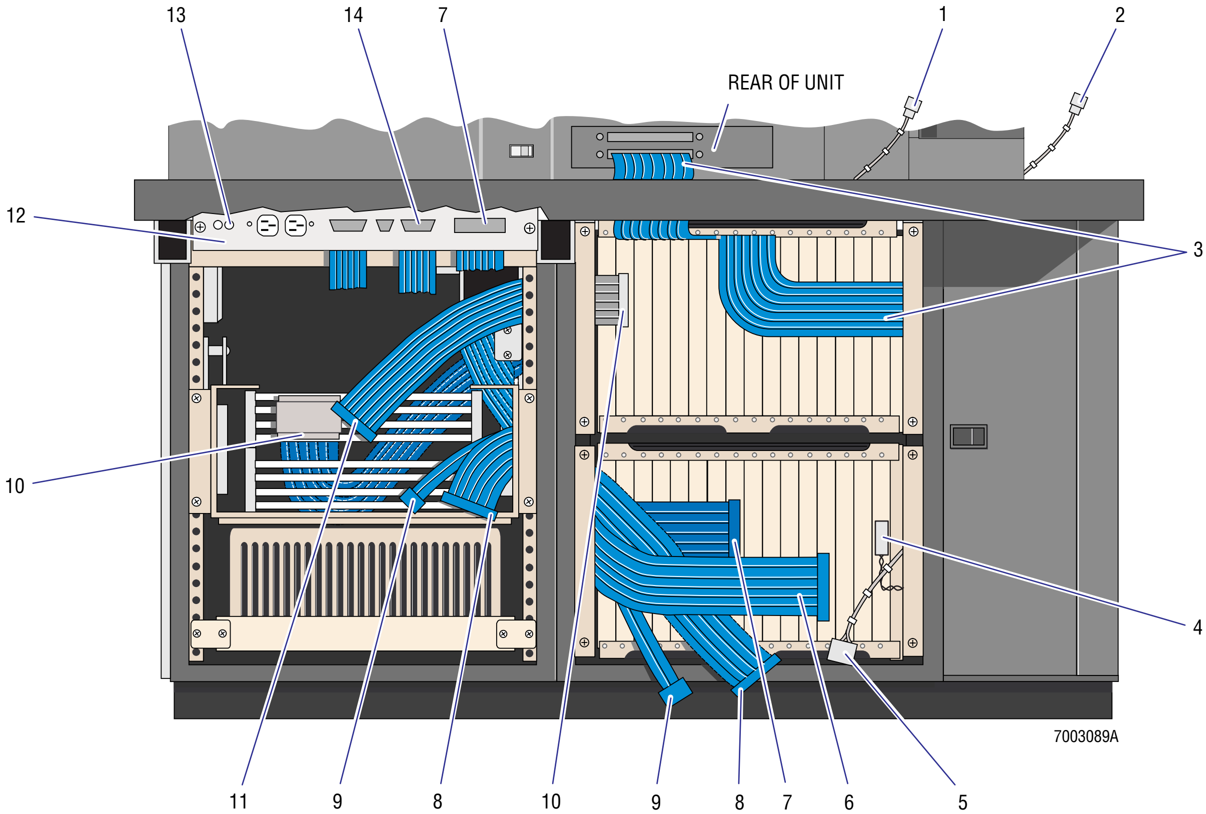


Table 8.2-10 Elite and Elite Analyzer, Interconnections, Detail of Parts (See Figure 8.2-10)

Item	Part Number	Description	Item	Part Number	Description
1	6028210	Backflow cable	8	6028022	Bitmap Memory card cable
2	6028374	Analyzer card (W52) cable	9	6028023	Scope signal cable
3	6028020	Sensor interface cable	10	6028038	Data Acquisition card cable
4	6028018	Sort transistor cable	11	6028024	CPU Pneumatics card cable
5	6028040	±36 V (W19) cable	12	6858355	Connector panel, Elite Analyzer
6	6856953	Cable harness (W12)	13	6028070	BNC (W31) cable
7	6028025	Data Lister Out cable	14	6028021	Serial communication cable

Figure 8.2-11 Multibus Card Cage, All Configurations (See Table 8.2-11)

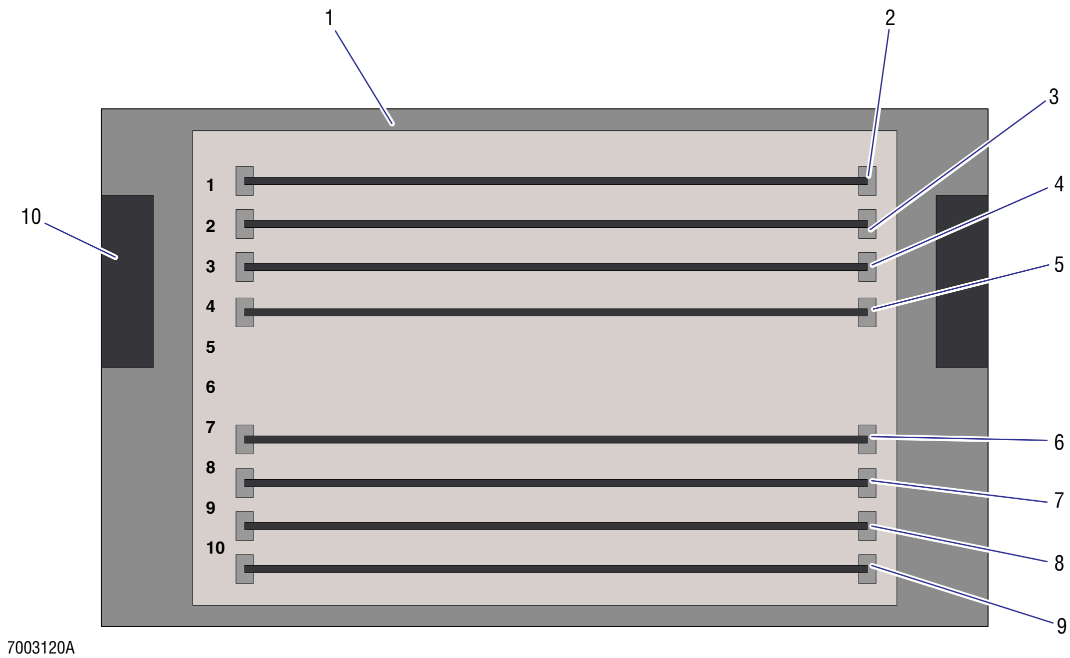


Table 8.2-11 Multibus Card Cage, All Configurations, Detail of Parts (See Figure 8.2-11)

Item	Part Number	Description
1	6704933	Computer backplane, Cytometer
2	6706571	Computer IBC-86C card (with on-board memory)* **Note:** If you are replacing an old Intel CPU 8086 card, PN 2016414, remove the 256K Memory card, PN 2016494 also. Once the new computer card is installed, the Cytometer Software must be downloaded from the Workstation.
3	6702664	Serial I/O card
4	6703761	Data Taker Interface card
5	7000681	External Memory card **Note:** Use only with the Computer IBC-86C card, PN 6706571.
6†	6704660	Camera Interface card
7	6704089	Dual CRT Control card
8	6703772	Digiscope card
9	6704463	Dual Laser Control card
10	2603033	Fan/blower, 24 Vdc, 73 CFM

* On units manufactured **after** 10/30/96.
† Elite Analyzer does not contain this component.

Figure 8.2-12 Data Acquisition Card Cage, Elite ESP (See Table 8.2-12

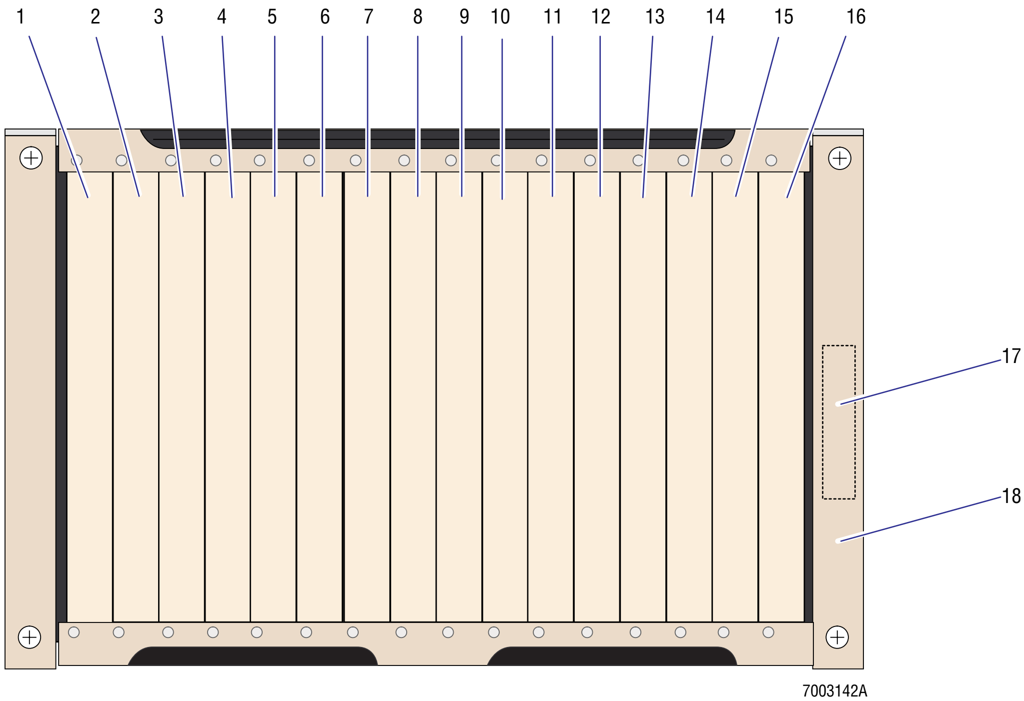


Table 8.2-12 Elite ESP Data Acquisition Card Cage, Detail of Parts (See Figure 8.2-12)

Item	Part Number	Description	Item	Part Number	Description
1	6704906	Pulse Generator and Clock card	10	6704142	Data Lister Cut card
2	N/A	Spare	11	6704185	Prism and Sort Window Test card
3	N/A	Spare	12	6704186 or 6706232	Bitmap and Sort Decision card Bitmap and Sort Decision R2 card
4	N/A	Spare	13	6704147	Interface and Scaler card
5	6705954	Pulse Pileup Det./TOF card	14	6704198	Sort Delay card
6	6704164	Mux and Scope Interface card	15	6704188	Sort Oscillator card
7	6704169	Quad PSH card 2	16	6704201	Sort Output card
8	6704169	Quad PSH card 1	17	6702608	Sort Transistor card
9	6704139	ADC and PSH control card	18	6704171	Data acquisition backplane

Figure 8.2-13 Cytometer, Inside Rear View (See Table 8.2-13

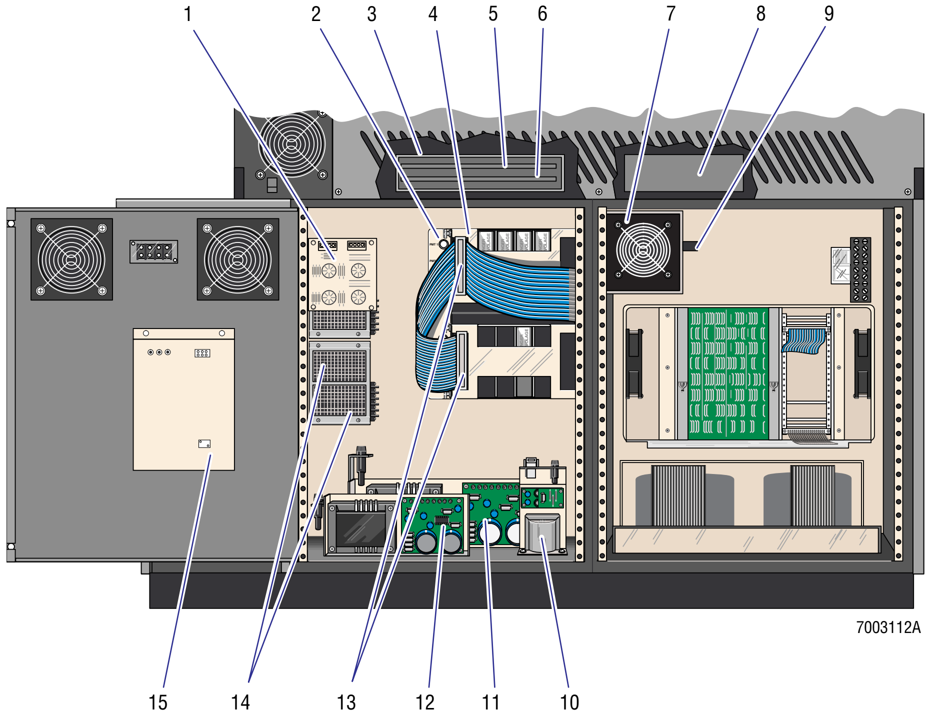


Table 8.2-13 Cytometer, Inside Rear View, Detail of Parts (See Figure 8.2-13)

Item	Part Number	Description
1	6704327	±36 V Regulator card
2	6856636	PMT power supply
3	6703950	HV DAC/PMT backplane
4	6804637	PMT (Bertan) HV Supply card
5	6704679	Motor Controller card
6	6705191	Autoclone Sorting Option Controller card
7	6857276	Fiber optics light source
8	4004074	Switcher power supply, 400W
9	3908025	Halogen lamp
10	6853275	15 V, 2 A power supply
11	6856870	15 V, 0.25A PMT power supply
12	6854358	15 V, 5 A power supply
13	6703941	PMT DAC R card
14	4004024	90 V power supply
15	6704960	Deflection power supply, HV, 3000 V

Figure 8.2-14 Cytometer, Right Rear Door, Inside View (See Table 8.2-14)

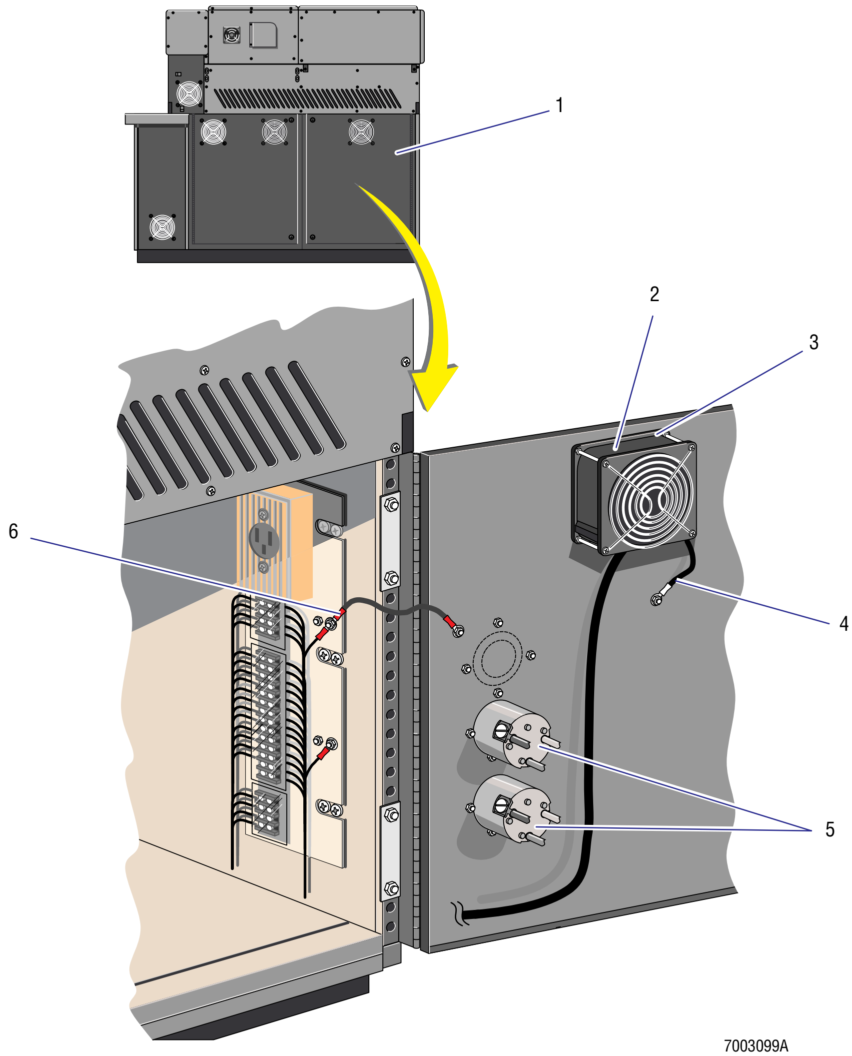


Table 8.2-14 Cytometer, Right Rear Door Inside View, Detail of Parts (See Figure 8.2-14)

Item	Part Number	Description
1	6856866	Transformer rack rear door
2	2603065	Fan blower, 112 CFM, 115 Vac
3	2510014	Shock mount
4	6028002	Cable assembly, ground
5	2105057	Power connector
6	6027967	Cable assembly, ground

Figure 8.2-15 Cytometer, Left Rear Door, Inside View (See Table 8.2-15)

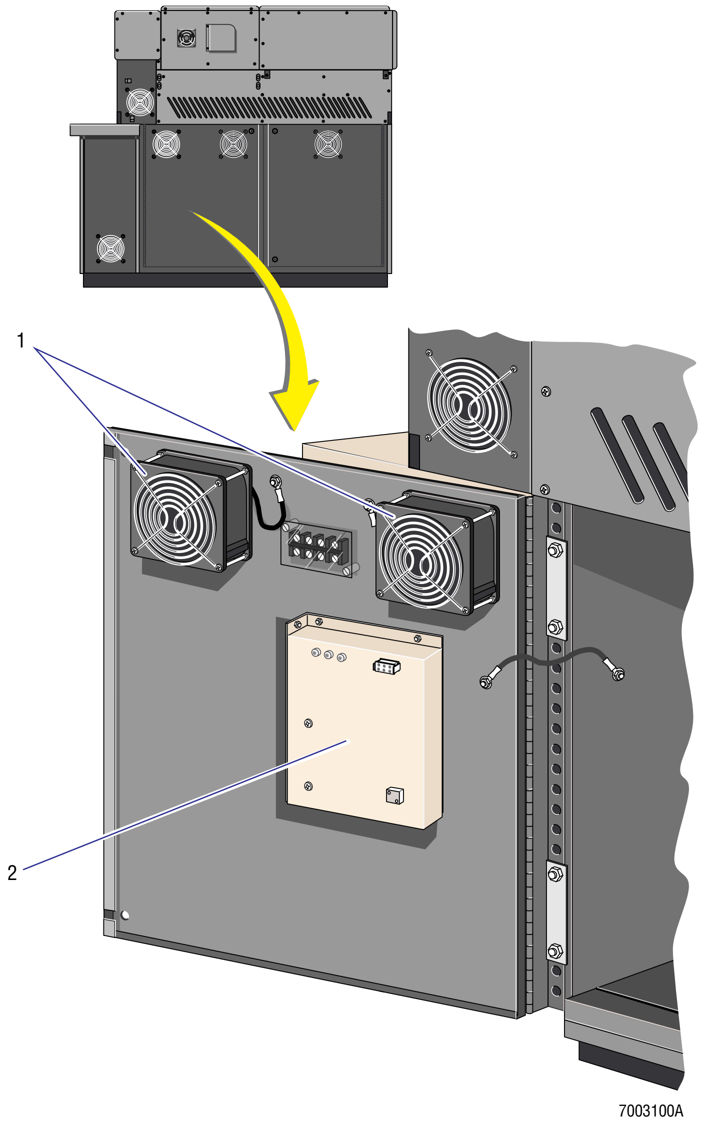


Table 8.2-15 Cytometer, Left Rear Door, Inside View, Detail of Parts (See Figure 8.2-15)

Item	Part Number	Description
1	2603065	Fan blower, 112 CFM 115 Vac
2	6705493	Deflection power supply, 3000 V

Figure 8.2-16 Cytometer, Left Front Corner, Inside View (See Table 8.2-16)

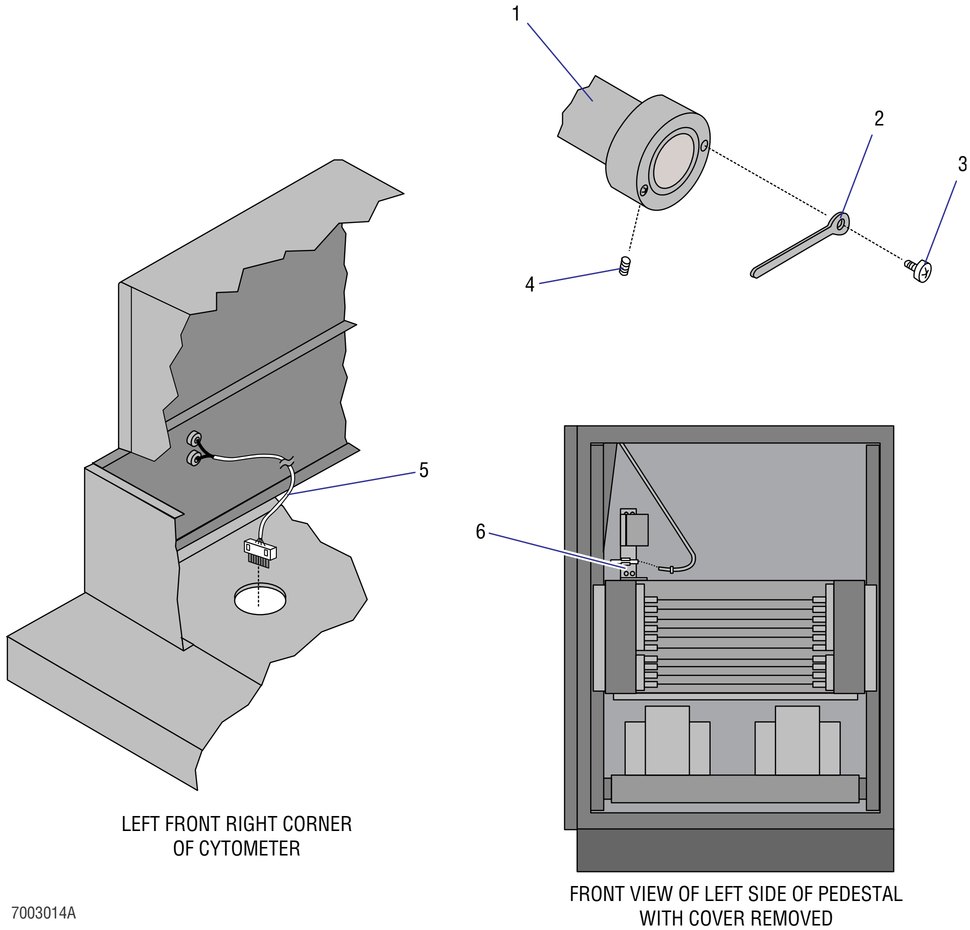


Table 8.2-16 Cytometer, Left Front Corner, Inside View (See Figure 8.2-16)

Item	Part Number	Description
1	2802006	Obscuration bar, jet-in-air
2	6857598	Jet-in-air ring
3	1019958	Screw, machine, 2-56 x 0.19
4	2807038	Setscrew, 6-32 x 0.250
5	6857636	Interlock bypass adapter
6	6857637	Interlock relay bracket

Figure 8.2-17 Analyzer Sample Drawer (See Table 8.2-17)

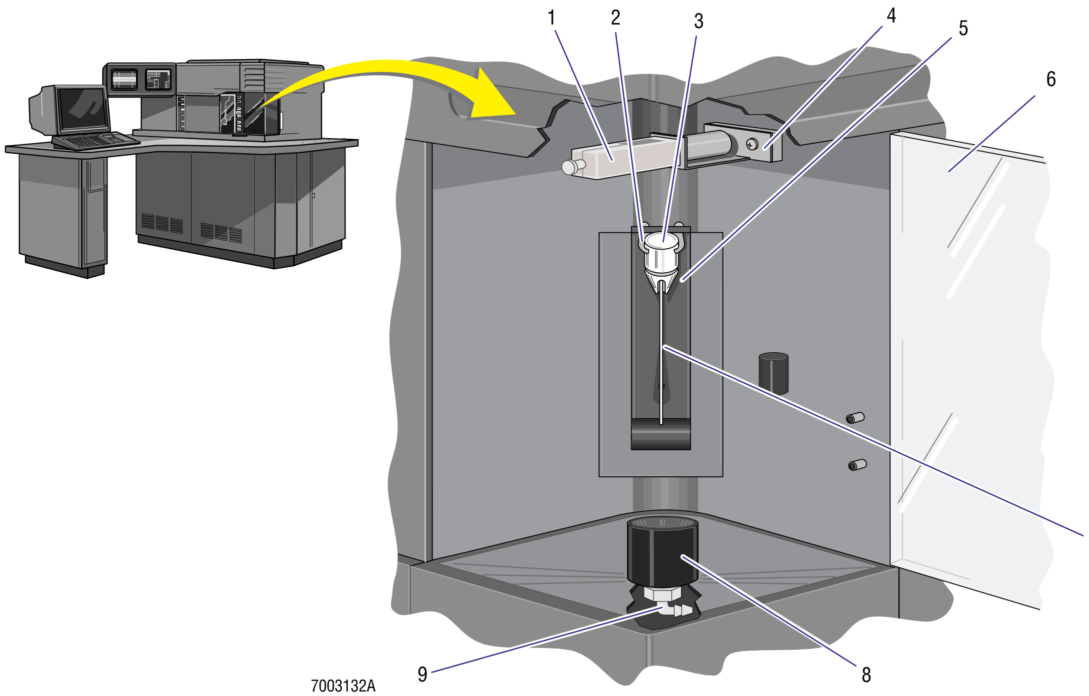


Table 8.2-17 Analyzer Sample Drawer, Detail of Parts (See Figure 8.2-17)

Item	Part Number	Description
1	1022054	Splash shield
2	6858923	Sample cap
3	3210004	Sample tubing, 0.010 i.d., insertion location
4	1018859	Pinch valve cover
5	1020913	Block, sample station
6	6858907	Door
7	1019224	Pickup probe
8	1017916	Base
9	6232205	Fitting, hose barbed, elbow, 0.125 i.d. to 10-32 thread

Figure 8.2-18 Analyzer, Top Inside View (See Table 8.2-18)

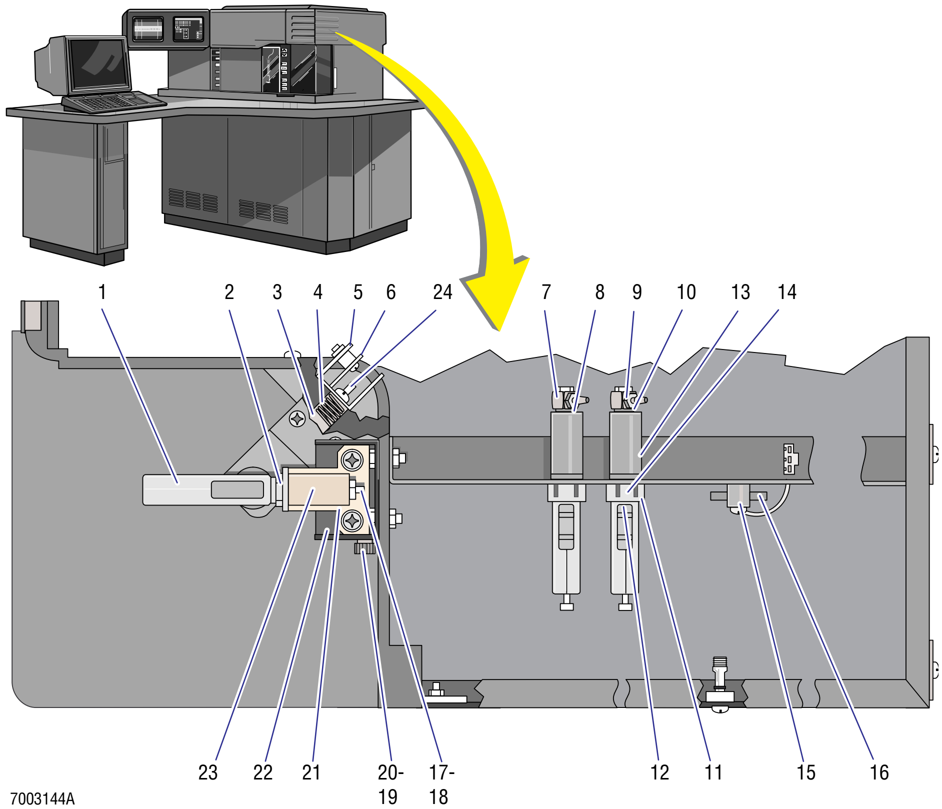


Table 8.2-18 Analyzer, Top Inside View, Detail of Parts (See Figure 8.2-18)

Item	Part Number	Description	Item	Part Number	Description
1	6803642	Double-acting pinch valve	13	6216012	Spacer, pneumatic, 0.562 o.d. x 0.500 i.d.
2	2822033	Nut, 47-32 UNS x 0.562 AFX	14	6211015	Pilot actuator
3	1018879	Plunger, test tube	15	6028086	Fluid detector cable
4	2515062	Spring, 0.344 o.d. x 0.62 x 0.25	16	2839043	Self-locking screw, 6-32 x 0.62
5	6858369	Sensor card mount	17	6232086	Union, hose barb, 0.062 i.d.
6	6703953	Pump motor detector	18	2523062	O-ring seal, 0.187 i.d.
7	6232205	Fitting hose barb, elbow, 0.125 i.d. to 10-32, nylon, white	19	2821010	Self-locking nut, 6-32 x 0.25 AFX
8	2523062	O-ring seal, 0.187 i.d.	20	2839039	Self-locking screw, 6-32 x 0.37
9	6232124	T-fitting, miniature, 10-32	21	1020901	Pinch valve bracket
10	6216345	Gasket, #10, black	22	6858370	Pinch valve mount bracket
11	1017501	Pinch valve mount	23	6211015	Pilot actuator
12	6855763	Standard pinch valve, pull apart			

Figure 8.2-19 Flow Cell Area, Elite (See Table 8.2-19)

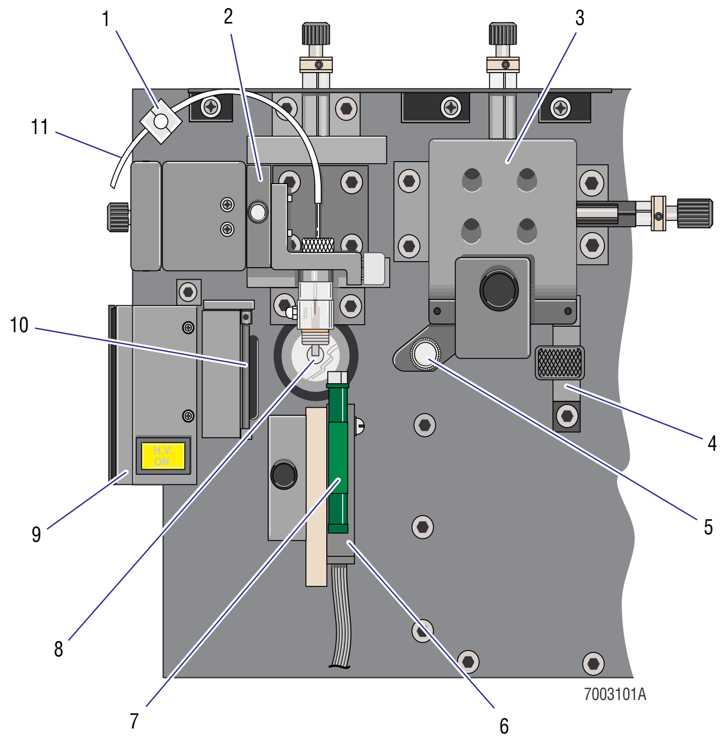


Table 8.2-19 Elite Flow Cell Area, Detail of Parts (See Figure 8.2-19)

Item	Part Number	Description
1	6857388	Double-acting pinch valve
	6211015	Pilot actuator
2	3814172	Flow cell stage
3	3814173	Beam stage
4	6857133	Shutter assembly
5	6856696	Beam shaper, 15x60 mm
	6857111	Beam shaper, 15x80 mm
6	6859007	Deflection body holder, new style
	6858222	Deflection body holder, old style
7	6704311	Deflection body, Autoclone Sorting Option
	6704754	Deflection body, standard
8	6859315	Pinhole snout 3X
9	6705402	Scatter sensor R2
	6705182	Peak scatter sensor
10	6857400	Prism holder
11	3210004	Sample tubing, 0.010 i.d.x 0.036 o.d.

Figure 8.2-20 Flow Cell Area, Elite Analyzer (See Table 8.2-20)

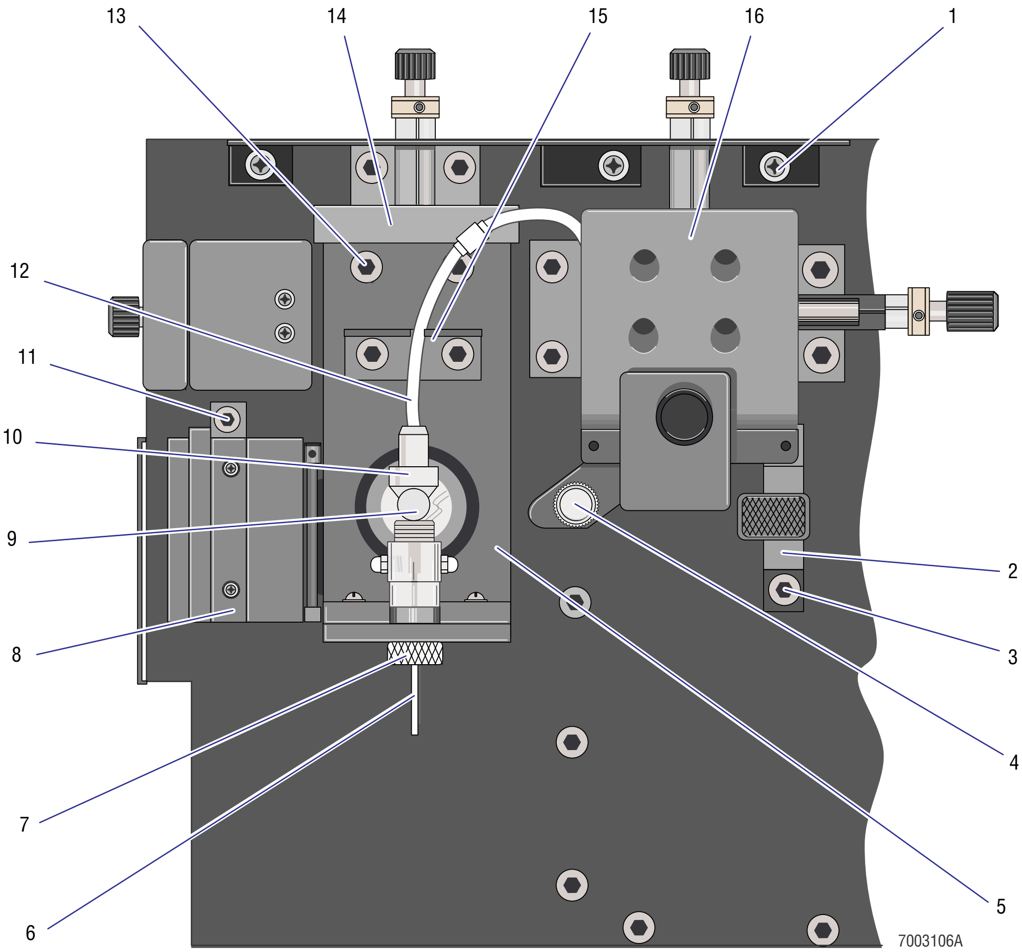


Table 8.2-20 Elite Analyzer Flow Cell Area, Detail of Parts (See Figure 8.2-20)

Item	Part Number	Description	Item	Part Number	Description
1	1019539	Interlock bracket	9	6856191	Flow cell tip
2	6857133	Shutter	10	6857066	Pinhole snout
3	2851558	Machine screw, 25-20 x 1.00	11	1016487	Sample tubing, 7.0
4	6858019 6857111 6856826	Beam shaper, 15x80 mm Beam shaper, 15x80 mm 2687 Beam shaper, 8x80 mm	12	2851836	Machine screw, 25-20 x 0.62
5	1019009	Flow cell bracket	13	3814172	X-Y-axis positioning stage
6	3210004	Pinch tubing, 0.010 i.d. x 0.036	14	1020903	Tube holder bracket
7	1010933	Nut clamp	15	3814173	X-Y-Z-axis positioning stage
8	6705597	Scatter sensor	16	N/A	Beam stage

Figure 8.2-21 Deflection Body and Holder (See Table 8.2-21)

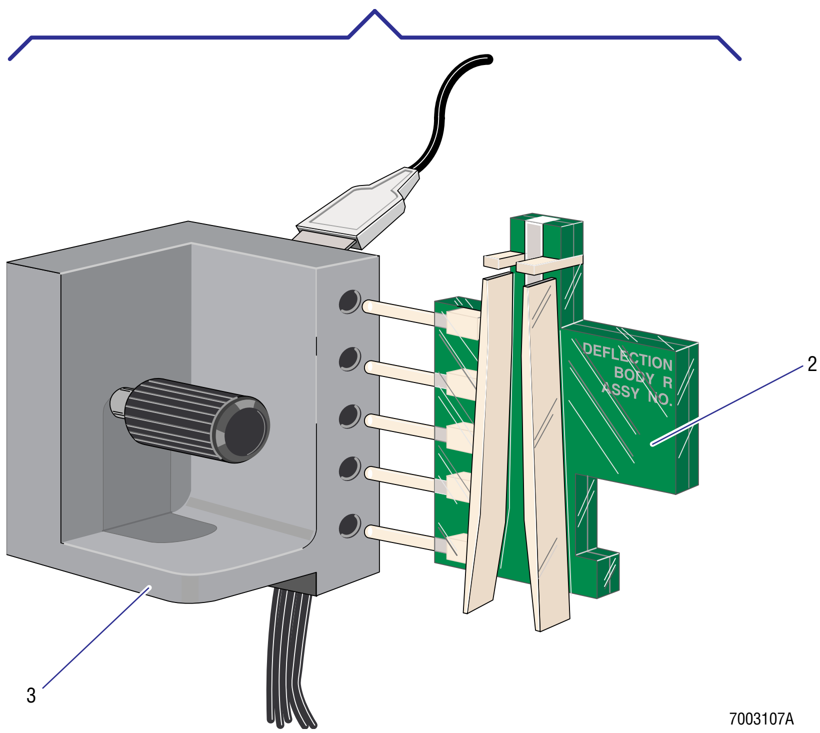


Table 8.2-21 Deflection Body, Detail of Parts (See Figure 8.2-21)

Item	Part Number	Description
1	6704754	Deflection body and holder
2	N/A	Deflection body holder
3	N/A	Deflection body

Figure 8.2-22 Photomultiplier Tubes (PMTs) (See Table 8.2-22)

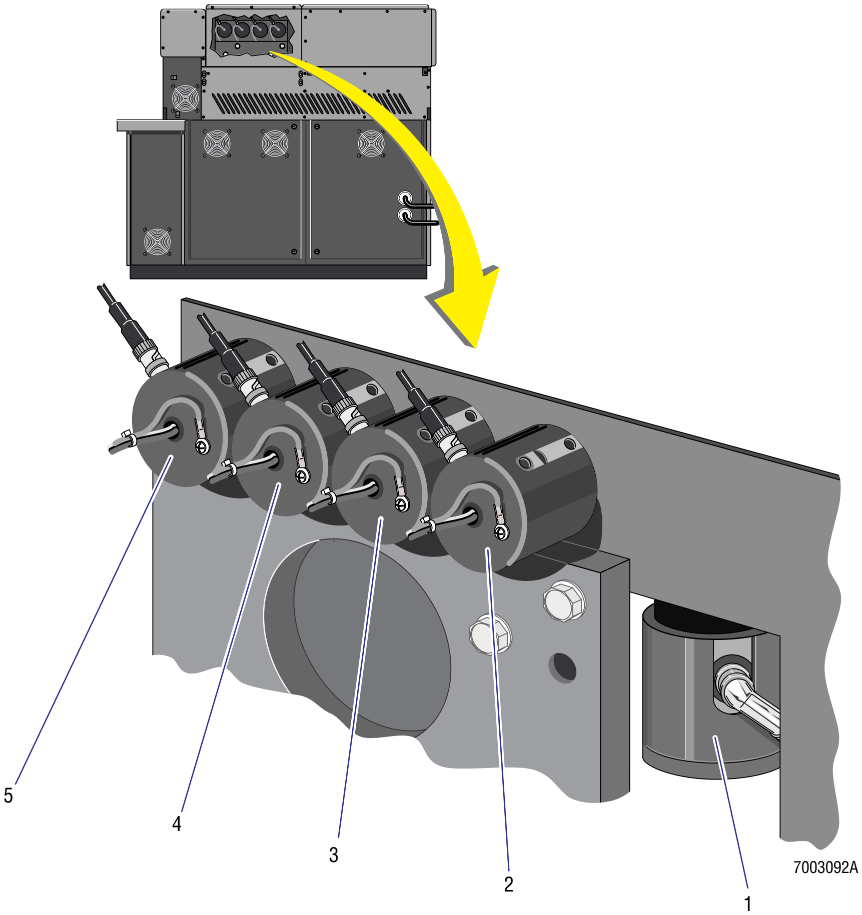


Table 8.2-22 Photomultiplier Tubes (PMTs), Detail of Parts (See Figure 8.2-22)

Item	Part Number	Description
1	6856505	PMT 4
2	6856833	PMT 5
3	6856833	PMT 3
4	6856833	PMT 2
5	6856833	PMT 1

Figure 8.2-23 Autoclone Sorting Option Mechanism (See Table 8.2-23)

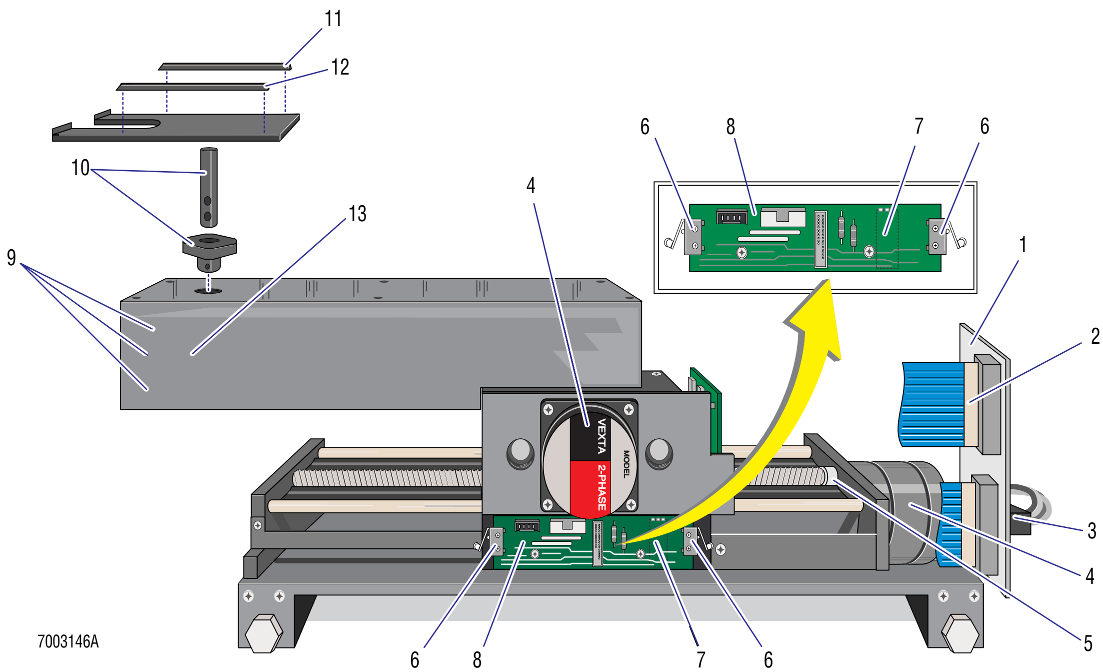


Table 8.2-23 Autoclone Sorting Option Mechanism, Detail of Parts (See Figure 8.2-23)

Item	Part Number	Description
1	6705328	Interconnect card
2	6028266	Flex cable
3	6028266	Main cable
4	3501018	Stepper motor
5	2508022	Shaft coupling, 0.188 and 0.250 i.d. ends
6	5103013	Snap-action switch
7	4836701	Opto switch
8	6705329	Position Detection card
9	4818006	Opto switch, 0.1 gap
10	6858187	Tray support
11	1018721	Tray cover
12	1020199	Tray
13	3505035	Stepper motor, 1.8 degree, 5.5 Vdc

Figure 8.2-24 Autoclone Sorting Option, Beak Waste Catcher (See Table 8.2-24)

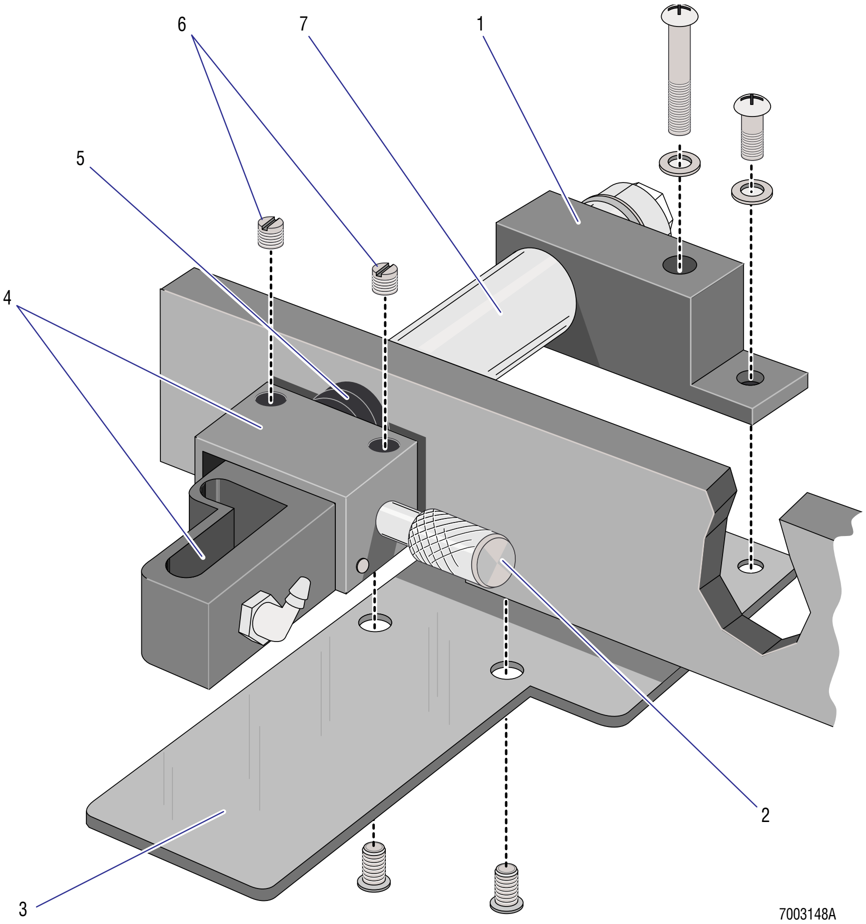


Table 8.2-24 Autoclone Sorting Option, Beak Waste Catcher, Detail of Parts (See Figure 8.2-24)

Item	Part Number	Description
1	1022047	Cylinder holder
2	1022048	Shaft, Autoclone Sorting Option beak
3	1022049	Plate, Autoclone Sorting Option beak
4	6858880	Autoclone Sorting Option beak
5	1018230	Beak actuator bellows
6	1007908	Threaded pin
7	6232037	Air cylinder, single-acting

Figure 8.2-25 Autoclone Sorting Option, Location and Connections (See Table 8.2-25)

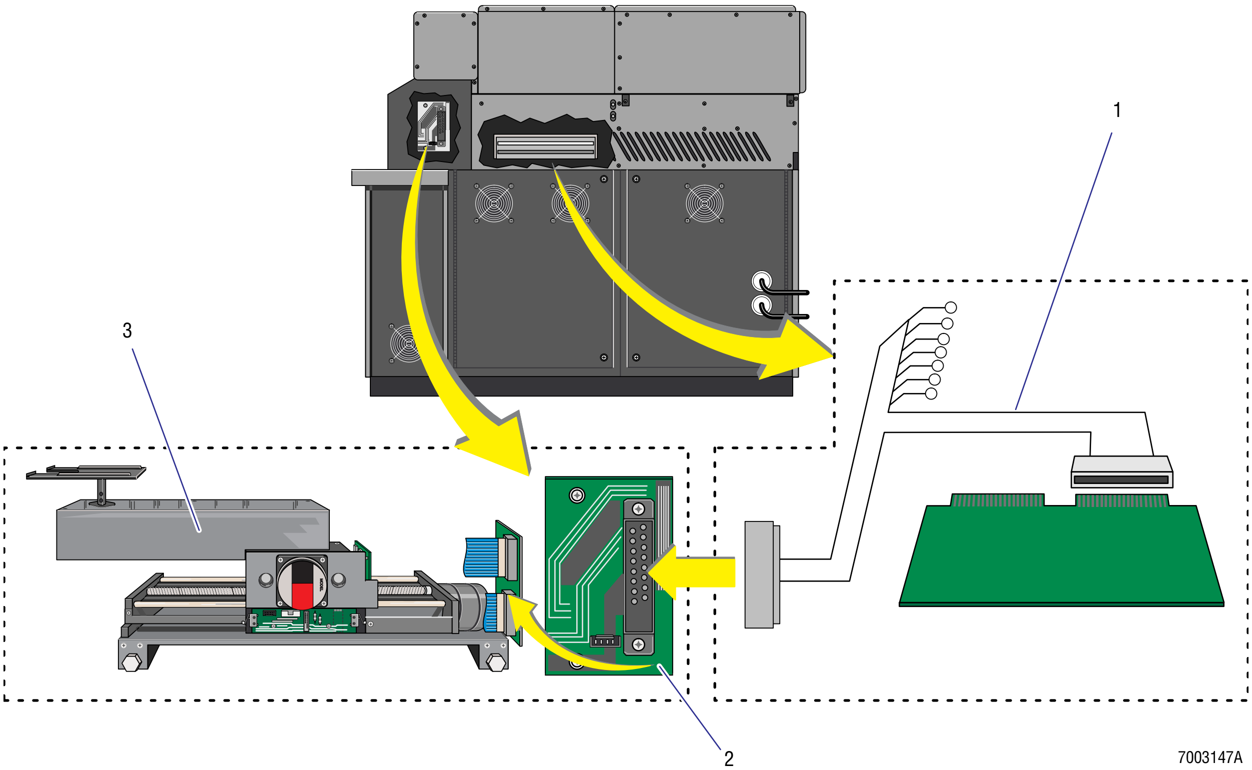


Table 8.2-25 Autoclone Sorting Option, Location and Connections, Detail of Parts (See Figure 8.2-25)

Item	Part Number	Description
1	6028268	Main cable
2	6705191	Autoclone Sorting Option card
3	6704313	Autoclone Sorting Option mechanism

Figure 8.2-26 Autoclone Sorting Option Accessories (See Table 8.2-26)

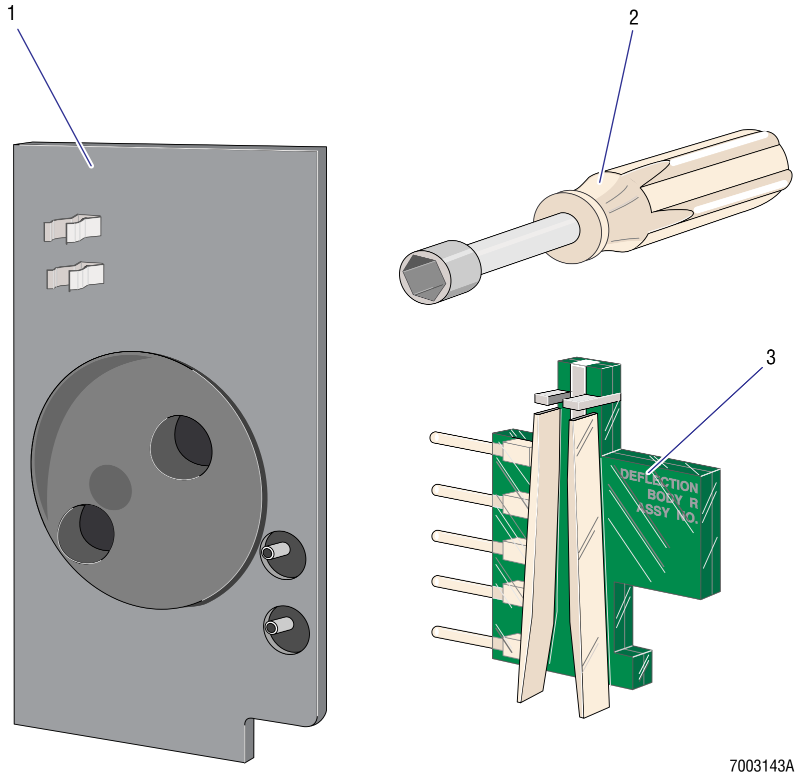


Table 8.2-26 Autoclone Sorting Option Accessories, Detail of Parts (See Figure 8.2-26)

Item	Part Number	Description
1	6856635	Cover bezel
2	5415377	Modified socket
3	6704311	Deflection plate

Figure 8.2-27 Transformer Drawer Assembly (See Table 8.2-27)

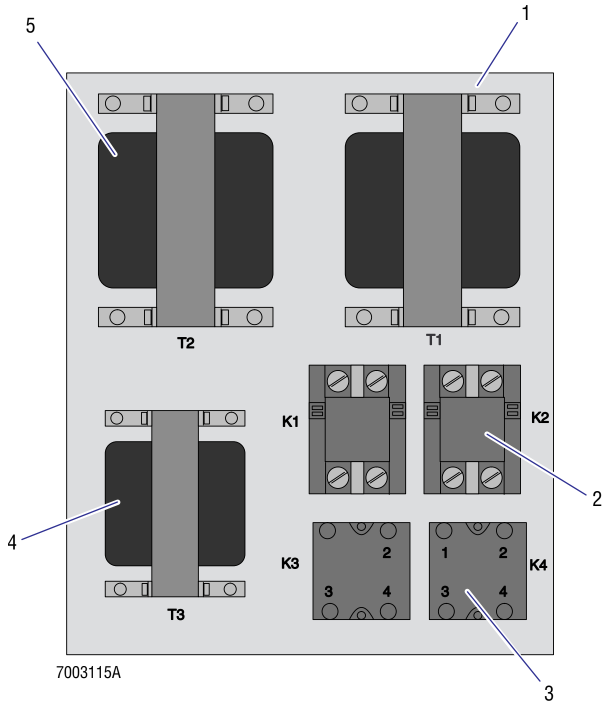


Table 8.2-27 Transformer Drawer Assembly, Detail of Parts (See Figure 8.2-27)

Item	Part Number	Description
1	6856911	Transformer drawer assembly
2	4506029	K1 and K2 relay, 2 pole, 120 Vac
3	4508007	K3 and K4 relay, 100-140 Vac, 10A, 3-32 Vdc input
4	5609034	T3 transformer, 100/220/240, 118 Vac, 50/60 Hz
5	5609035	T1 and T2 transformer, 100/120/240, 115 Vrms

Figure 8.2-28 Computer Box Assembly (See Table 8.2-28)

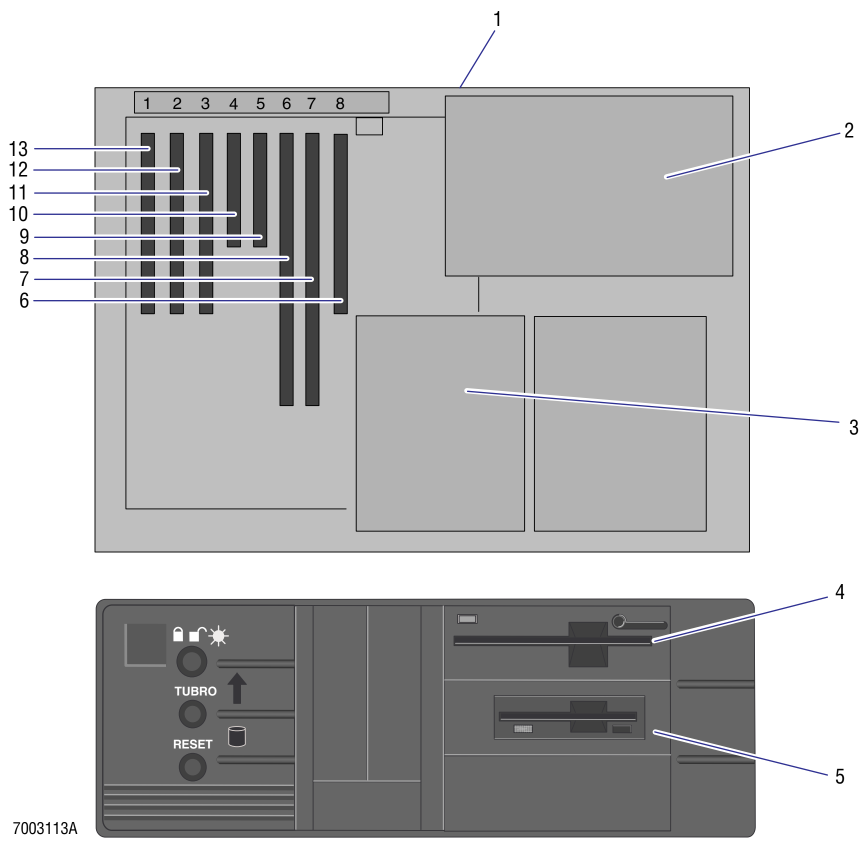


Table 8.2-28 Computer Box Assembly, Detail of Parts (See Figure 8.2-28)

Item	Part Number	Description	Item	Part Number	Description
1	6857479	Computer box assembly	8	N/A	Spare
2	7260017	Power supply, 220 W	9	6028212	Cable from slot 4
3	2016416	Hard drive, non-removable	10	2016390	2-channel RS-232 Serial card
4	2016298	Floppy drive, 5.25 in.	11	2016413	V-RAM VGA card
5	2016328	Floppy drive, 3.50 in.	12	N/A	Spare
6	2016424	Disk Controller card	13	6704665	Lister AB card
7	2016378	Memory Expansion card, 2 MB			

Figure 8.2-29 Beam Splitter (Dichroic) (See Table 8.2-29)

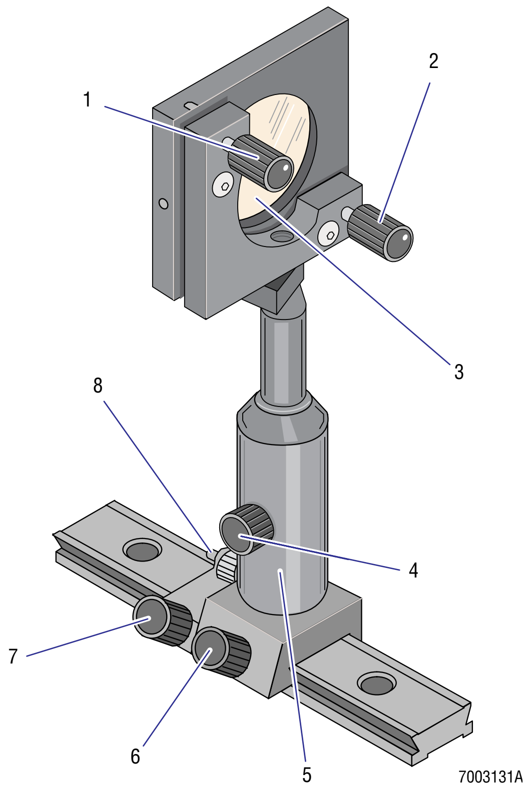


Table 8.2-29 Beam Splitter (Dichroic), Detail of Parts (See Figure 8.2-29)

Item	Part Number	Description
1	N/A	Vertical rotation knob
2	N/A	Horizontal rotation knob
3	3814223	Adapter, 45D mirror
4	N/A	Height setscrew
5	6857634	Beam splitter stage
6	N/A	Rail setscrew
7	N/A	Fine adjustment setscrew
8	N/A	Fine adjustment knob

Figure 8.2-30 Beam Shaper (See Table 8.2-30)

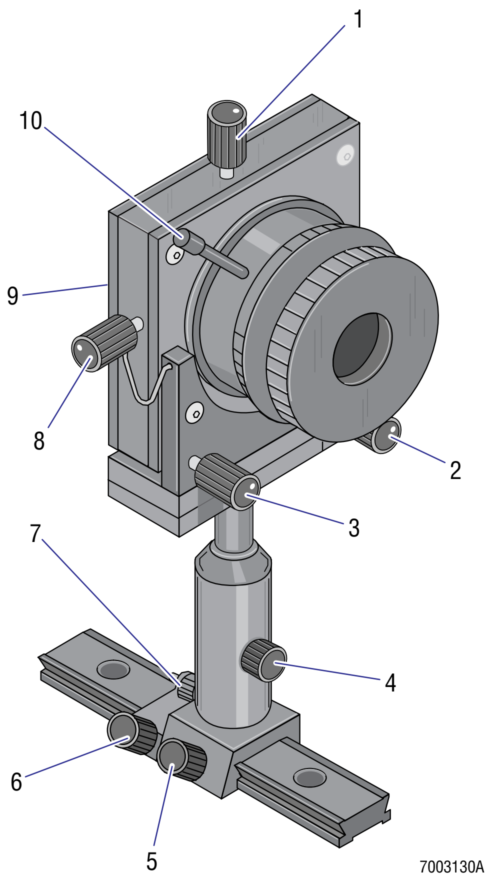


Table 8.2-30 Beam Shaper, Detail of Parts (See Figure 8.2-30)

Item	Part Number	Description
1	N/A	Vertical translation knob
2	N/A	Horizontal rotation knob
3	N/A	Vertical rotation knob
4	N/A	Height setscrew
5	N/A	Rail setscrew
6	N/A	Fine adjustment setscrew
7	N/A	Fine adjustment knob
8	N/A	Horizontal translation knob
9	N/A	Focus ring
10	N/A	Lateral translation knob

Figure 8.2-31 Flow Cell Stage, Elite ESP (See Table 8.2-31

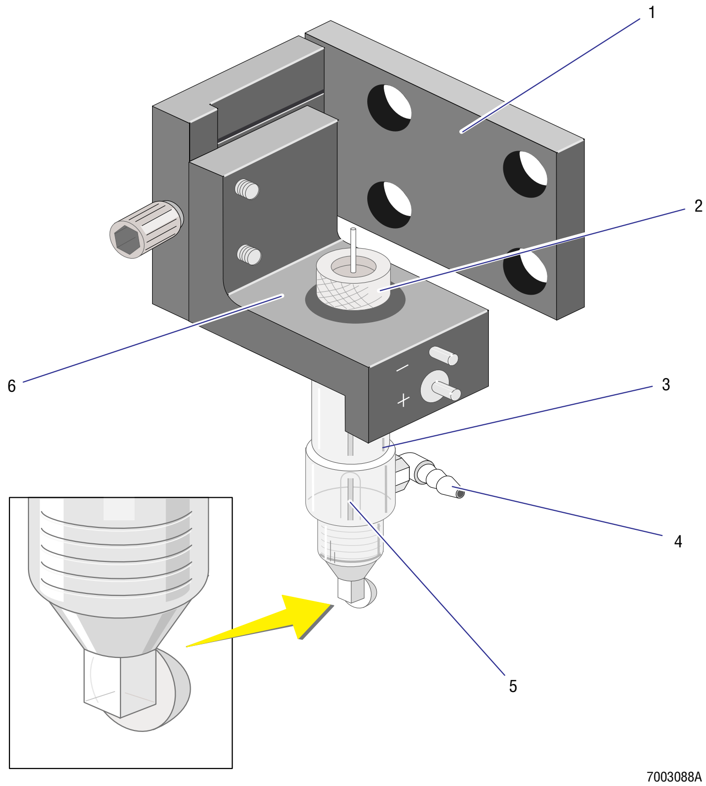


Table 8.2-31 Elite ESP Flow Cell Stage, Detail of Parts (See Figure 8.2-31)

Item	Part Number	Description
1	3814271	Ultra-compact positioner
2	1010932	Upper nut, insertion rod gland nut
3	6856762	Flow cell body, high optical
4	6232208	Hose barb fitting, elbow, 0.093 i.d., nylon, white
5	6859397	Sort sense, 140 μ
	6859300	Flow cell tip, 100 μ 3X
	6859313	Flow cell tip, 76 μ 3X
6	6858368	Flow cell bracket

Figure 8.2-32 Flow Cell Stage, Elite (See Table 8.2-32)

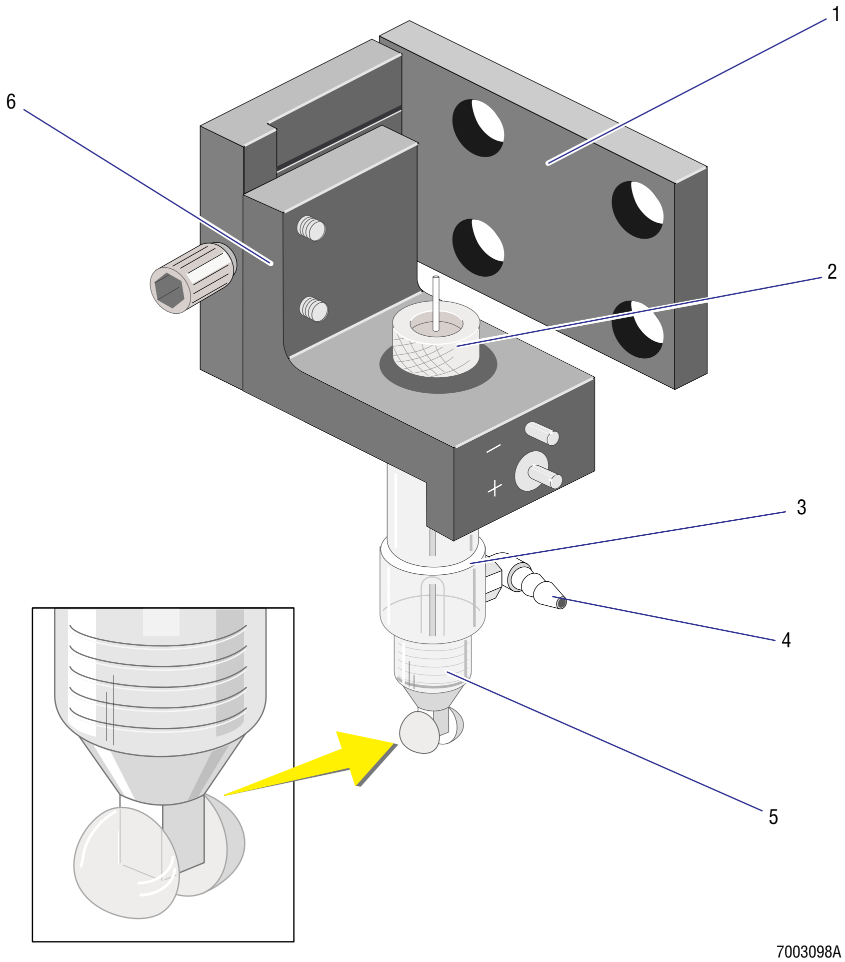


Table 8.2-32 Elite Flow Cell Stage, Detail of Parts (See Figure 8.2-32)

Item	Part Number	Description
1	3814271	Ultra-compact positioner
2	1010932	Upper nut, insertion rod gland nut
3	6856762	Flow cell body, high optical
4	6232208	Hose barb fitting, elbow, 0.093 i.d., nylon, white
5	6602642	Biosense flow cell
6	6858368	Bimorph flow cell bracket

Figure 8.2-33 Model 74 HeCd Laser Mounting Hardware (See Table 8.2-33)

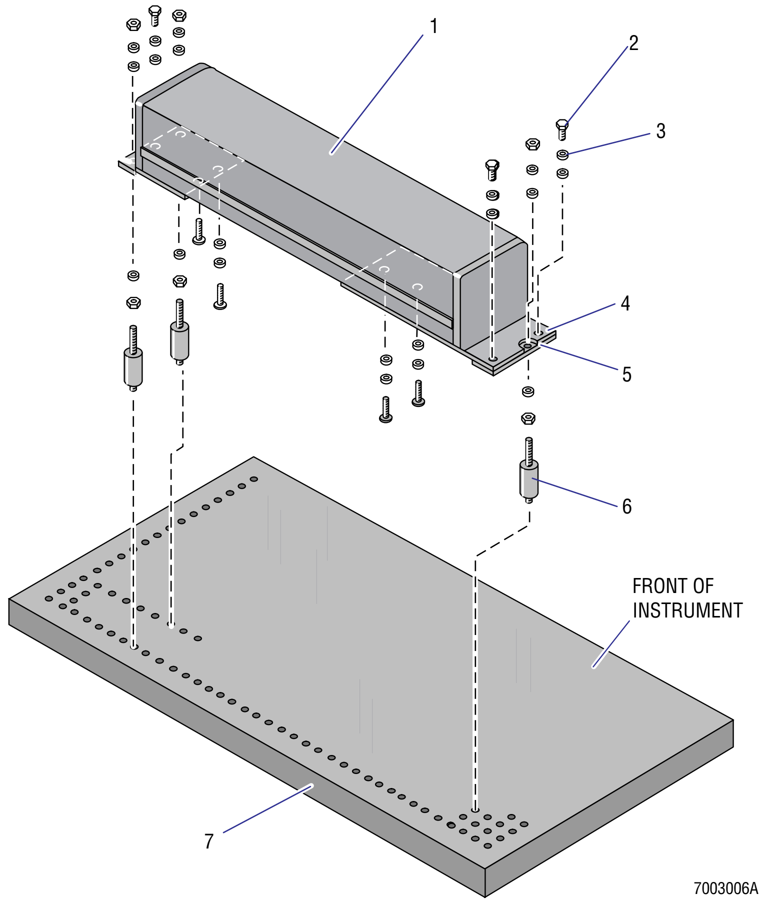


Table 8.2-33 Model 74 HeCd Laser Mounting Hardware, Detail of Parts (See Figure 8.2-33)

Item	Part Number	Description
1	6705218	HeCd 74 Laser and Head, tested
2	1022190	Screw, 0.25 x 0.28 x 0.94
3	2523656	Washer, 0.25
4	1017375	HeCd mounting back plate
5	1017262	Plate bottom cover
6	1016827	Plate support, upright rear
7	1017261	HeCd mounting front plate

Figure 8.2-34 Argon Laser Mounting Hardware (See Table 8.2-34)

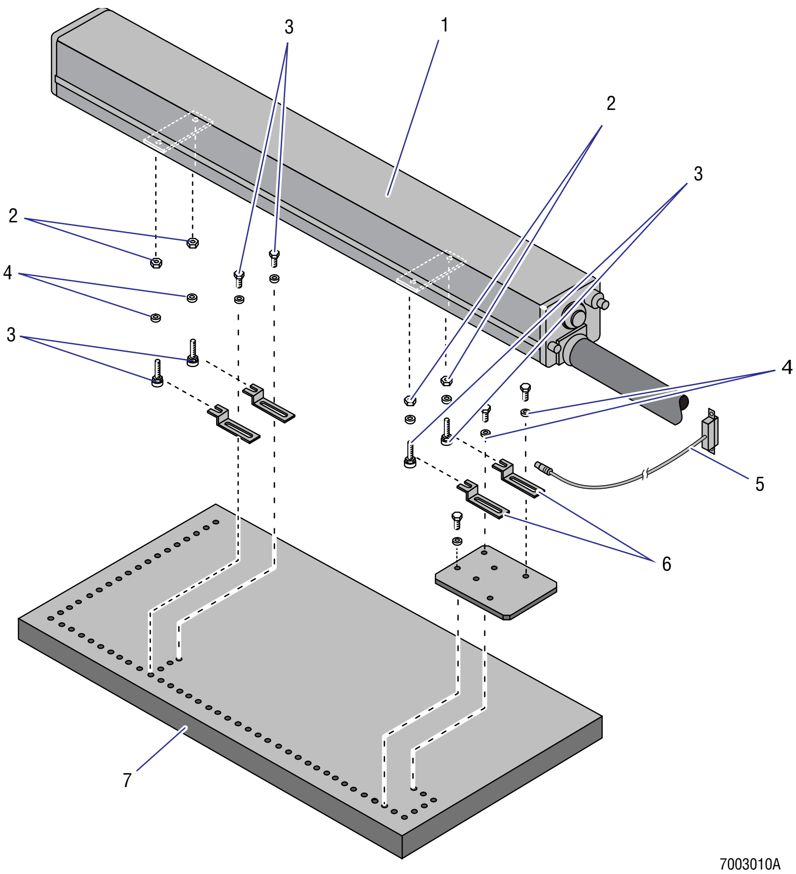


Table 8.2-34 Argon Laser Mounting Hardware, Detail of Parts (See Figure 8.2-34)

Item	Part Number	Description
1	3814232	Argon Ion system laser
2	2851598	Hex nut, 37-16
3	2851762	Machine screws, metric, M6x25 mm
4	2826051	Washer, split-lock
5	6028218	Cable assembly with interlock
6	1020271	Laser mounting brackets and support plate
7	3814233	Optical table
8	2851762	Machine screws, metric, M6x25 mm

Figure 8.2-35 HeNe Laser Mounting Hardware (See Table 8.2-35)

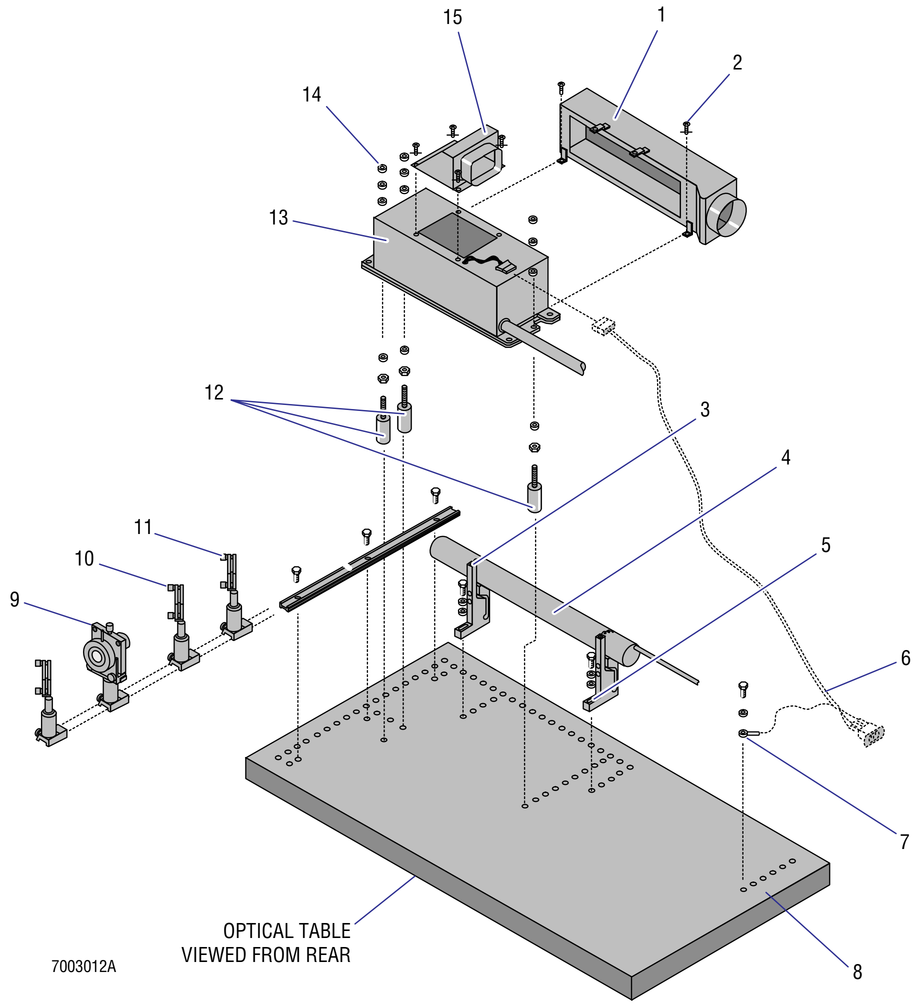


Table 8.2-35 HeNe Laser Mounting Hardware, Detail of Parts (See Figure 8.2-35)

Item	Part Number	Description	Item	Part Number	Description
1	6857424	Duct plenum	9	6912985	Beam reducer/expander
2	1019658	Air duct bracket	10	6857611	Beam splitter, Argon 488
3	2523549	Spring, 0.25 o.d.	11	6857612	Beam splitter, HeNe 633
4	6857515	HeNe Laser	12	6856681	Argon mounting upright
5	6857112	HeNe mounting feet	13	6857516	Argon laser
6	6028016	Laser control (W49) cable	14	2814004	Screw, 25-20 x 0.50
7	6011002	Wire tie, 6.7 x 0.14, nylon	15	6857347	Laser intake duct
8	N/A	Mounting plate			

Figure 8.2-36 Laser Placement, Top View (See Table 8.2-36)

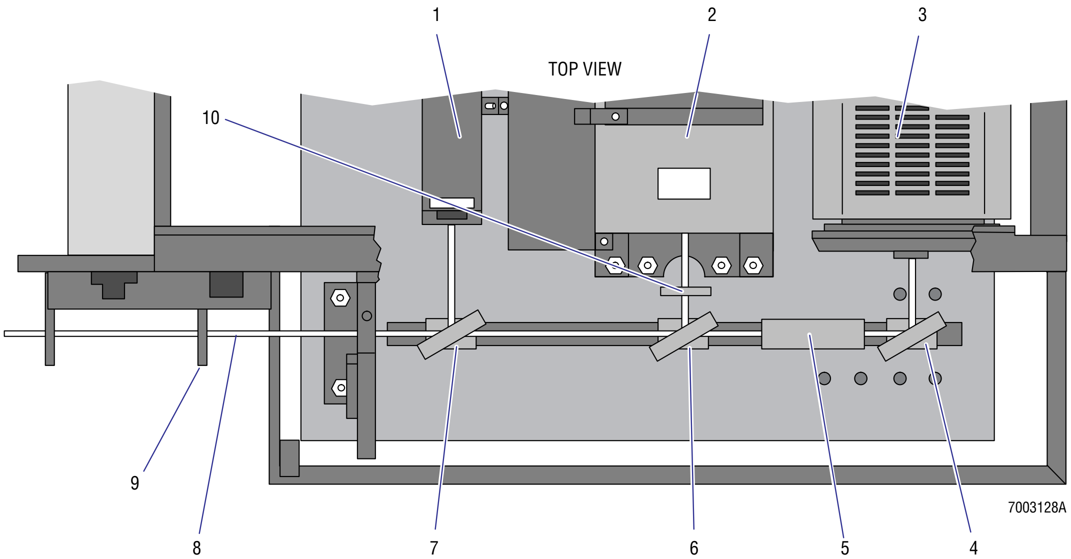


Table 8.2-36 Laser Placement, Top View, Detail of Parts (See Figure 8.2-36)

Item	Part Number	Description
1	6912749	Red HeNe laser (laser 2)
2	6912750	Argon laser (laser 1)
3	6912792	Innova 305, Coherent, laser
4	3814286	325 mirror (reflects 325 to 365 nm)
5	6856298	Beam reducer/expander (part of kit PN 6912946)
6	3814223	488 mirror (reflects 488 nm)
7	3814222	633 mirror (reflects 633 nm)
8	N/A	Laser beam
9	6857236	Small target
10	6858459	3-hole multipositional target

Figure 8.2-37 Cytometer, Inside Right Rear View (See Table 8.2-37)

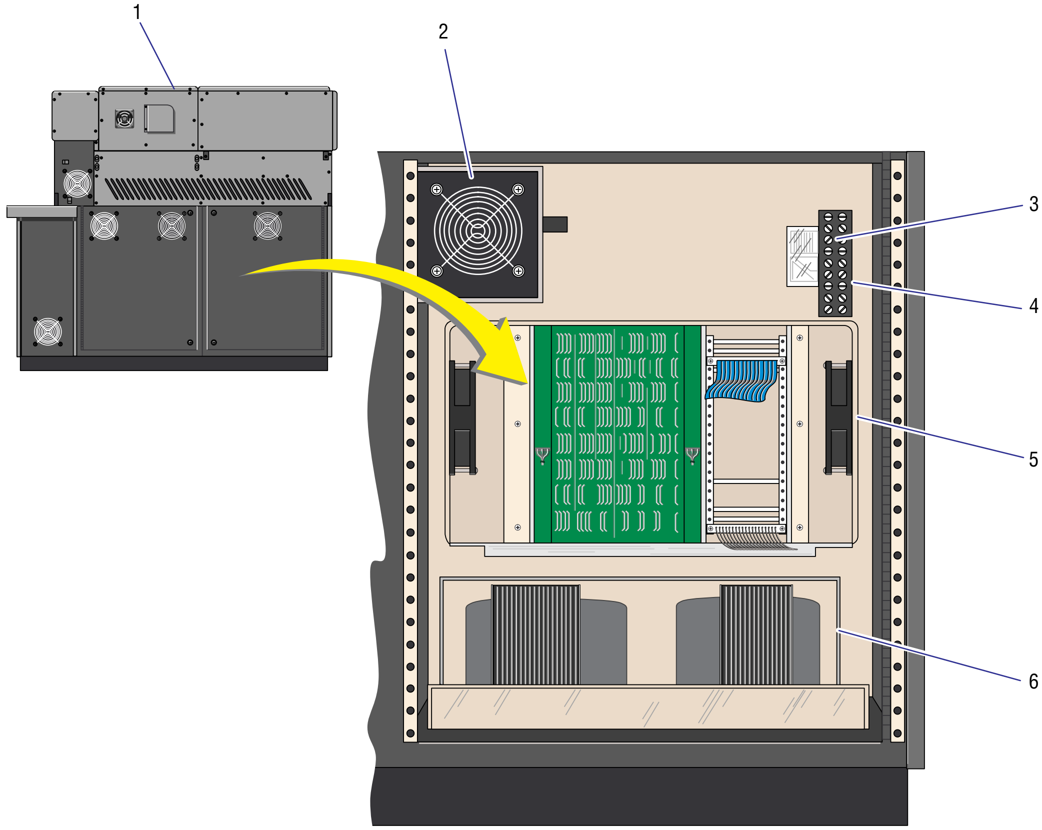


Table 8.2-37 Cytometer, Inside Rear View, Detail of Parts (See Figure 8.2-37)

Item	Part Number	Description
1	6857604	Cover, top, removable
2	2603044	Fan/blower, 200 CFM, 115 Vac
3	6028016	Cable, laser controller
4	6857637	Laser interlock relay bracket
5	6704933	Cytometer card cage
6	6856911	Transformer drawer

Figure 8.2-38 Omnichrome HeCd Laser (See Table 8.2-38)

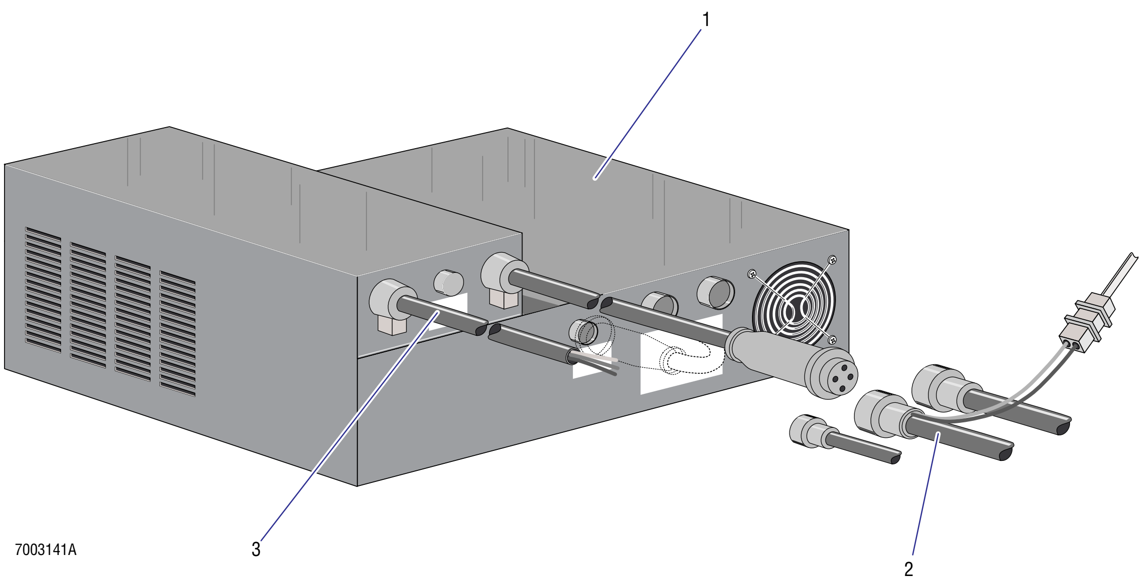


Table 8.2-38 Omnichrome HeCd Laser (See Figure 8.2-38)

Item	Part Number	Description
1	6912834	HeCd 74 laser **Note:** Use PN 3814244 for head and power supply, 325 nm, 30 mW.
2	6028256	Interlock cable
3	6027225	Line cord, 3-18 ga.

ILLUSTRATIONS

TABLES

A.1 TOLERANCES AND LIMITS

Pressure

Compressor Module

System pressure - 30 psi
Compressor pressure - 50 psi

House Air

System Pressure Gauge - must not exceed 30 psi
System vacuum gauge should read at least 10 in. Hg.

Pneumatics

Sheath pressure - 12 psi
Sample pressure - 12 psi

Switches

DVM: set to DC V.
DVM: 4 to 5 V immediately after you turn instrument on; 0 V after Compressor module pressure builds up.

Initial Installation Settings

Argon Laser

Light output - 20 second delays

Camera

Zoom setting minimum:

- Newer camera assemblies - 1.0
- Older camera assemblies - 0.7.

Front and Back Porch Settings for Best Streams

Non-ESP systems, adjust the Sort Oscillator card to make the front and back porch adjustments:

- Adjust R93 for side streams. Keep the front porch level between 60% and 95% of the main pulse.
- Adjust R92 for center (waste) stream. Keep the back porch level between 5% and 30% of the main pulse.

Test Points

Pneumatic Interface Card

Table A.1-1 Pneumatic Interface Card Adjustments

Adjustment on Card	Adjustment Range	Test Point
R31	+10.00 ±0.01 Vdc	TP5
R34	-10.00 ±0.01 Vdc	TP1
R81	-10.00 ±0.01 Vdc	TP10
R54	+10.00 ±0.01 Vdc	TP3
R51	+8.00 ±0.01 Vdc	TP2

Power Supplies

Test the Deflection power supply:

J2-Black = Ground
J3-Blue = -2.3 to 3.0 Vdc
J4-Red = +2.3 to 3.0 Vdc

Optics

Beam Expander

Size of beam entering beam expander - approximately 0.3 mm in diameter.
Size of beam exiting the beam expander - approximately 0.15 mm in diameter before passing through the HeNe mirror.

Signal Adjustments

Beam Translator Upgrade

Adjust between the peak of the Argon-caused signal and the peak of the UV-caused signal:

20 microseconds for a 100 µ tip
30 microseconds for a 76 µ tip.

Adjust between the peak of the UV and HeNe and the Argon-caused signals for time separation of:

40 microseconds for the 100 µ sort sense tip at 12 psi, or
60 microseconds for the 76 µ sort sense tip at 12 psi, or
7 microseconds for the 76 µ jet-in-air flow cell tip.

Gated Amp Laser Alignment (Time/Space Separated Laser Alignment)

Adjust between the peak of the HeNe laser-generated signal and the peak of the UV laser-generated signal by adjusting the HeNe laser dichroic on the optical rail:

20 microseconds for a 100 µ tip.
30 microseconds for 76 µ tip.

Adjust between the peak of the Argon laser-generated signal and the peak of the UV laser-generated signal by adjusting the Argon dichroic on the optical rail.

20 microseconds for a 100 µ tip
30 microseconds for a 76 µ tip.

Time separation between the peak of the UV and HeNe laser-generated signals and the Argon laser-generated signals:

40 microseconds for the 100 µ sort sense tip at 12 psi
60 microseconds for the 76 µ Sort Sense tip at 12 psi
7 microseconds for the 76 µ jet-in-Air tip.

A.2 JUMPERS AND SWITCH SETTINGS

Jumpers for Circuit Cards Used During Installation and Upgrade Procedures

3 PMT Sub SW-R2 Card

For 5 PMT installation:

E3-E4 In
E1-E2 Out
E5-E6 Out

HV DAC Card

Jumper to be HV DAC 2 card for PMT 5 installation.

E5 - E9
E6 - E10
E7 - E11
E8 - E12

HV DAC Control Card

E1-E5
E2-E6
E3-E7
E4-E8.

HV DAC Control Card (Gated Amp, 5 PMT option)

E5-E9
E6-E10
E7-E11
E8-E12.

Lister AT Card on PC Model 486

E71-E72

Mux and Scope Card

For Gated Amp upgrade:

E1-E2
E3-E4

Sensor Interface Card

For Watchdog timing procedure:

Remove E25-E26.

Jumpers for Cards in Card Cages

Multibus Card Cage

Table A.2-1 Multibus Card Cage Jumpers and Switch Settings

<table>
<tr><th>Card</th><th>Jumper</th></tr>
<tr><td>CPU IBC 86C</td><td>Intel factory configured</td></tr>
<tr><td>Serial I/O</td><td>none</td></tr>
<tr><td>Data Taker Interface</td><td>none</td></tr>
<tr><td>256K Memory card

Note: If the instrument was manufactured after 10/30/96 or if the instrument has the new computer card (PN 6706571), see the jumper settings for the External Memory card.</td><td>
<table>
<tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th></tr>
<tr><td>SW1</td><td>X</td><td>X</td><td>X</td><td>X</td><td></td><td></td><td></td><td></td></tr>
<tr><td>SW2</td><td>O</td><td>O</td><td>O</td><td>O</td><td>O</td><td>O</td><td>O</td><td>O</td></tr>
<tr><td>SW3</td><td>O</td><td>O</td><td>O</td><td>X</td><td>X</td><td>O</td><td></td><td></td></tr>
<tr><td>SW4</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>O</td><td></td><td></td></tr>
<tr><td>SW5</td><td>O</td><td>O</td><td>O</td><td>O</td><td>O</td><td>O</td><td></td><td></td></tr>
<tr><td>SW6</td><td>O</td><td>O</td><td>O</td><td>O</td><td>O</td><td>O</td><td></td><td></td></tr>
<tr><td>SW7</td><td>X</td><td>X</td><td>X</td><td>O</td><td>O</td><td>X</td><td></td><td></td></tr>
<tr><td>SW9</td><td>O</td><td>O</td><td>O</td><td>O</td><td></td><td></td><td></td><td></td></tr>
</table>
X = Closed
O = Open
E19 - E20 open
E25 - E26 jumpered</td></tr>
<tr><td>External Memory</td><td>X1 jumpered
X6 (a 3-pin jumper) jumpered to the +12 V position</td></tr>
<tr><td>Camera Interface</td><td>E19-E20, E21-E22, E37-E39, E38-E42, E41-E45, E44-E46</td></tr>
<tr><td>Dual CRT Control</td><td>E11-E12, E13-E14</td></tr>
<tr><td>Digiscope</td><td>E69-70,E31-39,E25-17
SW - ALL <u>ON</u>.</td></tr>
<tr><td>Dual Laser Control</td><td>E4-E7, E25-E26, E28-E29, E31-E32, E34-E35, E37-E38, E40-E41</td></tr>
</table>

Non-Gated Amp Card Cage

Table A.2-2 Non-Gated Amp Card Cage Jumpers

Card	Jumpers
Gated Amp Control R2	E4-E5, E7-E8
3PMT Sub Amp SW-R	E9-E11
Dual FL Amp SW-R	E8-E9, E11-E12
Scat/CV Amp SW-R	E9-E11,E5-E7
Peak Scatter/Mux SW-R	E1-E2,E3-E4,E5-E7
Sensor Interface	E2-E3, E5-E6, E8-E9, E11-E12, E13-E14, E16-E17, E19-E20, E22-E23

Gated Amp Card Cage

Table A.2-3 Gated Amp Card Cage Jumpers

Card	Jumpers
Gated Amp Control R3	None
Dual FL Amp SW-R	E8-E9, E11-E12
3 PMT Sub Amp SW-R2	E3-E4,E9-E11
3PMT Sub Amp SW-R1	E9-E11
PMT Gated Amp	None
Quad 20/40/60 Microsecond Delay 2	E1-E2, E3-E4, E10-E11 In
Quad 7 Microsecond Delay 2	E10-E15 In
#1 Quad 7 Microsecond Delay	E10-E11 In
Quad 20/40/60 Microsecond Delay 1	E1-E2, E3-E4, E9-E10 In
Scat/Aux Gated Amp	None
3PMT Sub Amp SW-R	E1-E2, E5-E6, E9-E11
Peak Scatter/Mux SW-R	E1-E2, E3-E4, E5-E7
Scat/CV SW R1	E9-E11, E5-E7
Sensor Interface	E2-E3, E5-E6, E8-E9, E11-E12, E13-E14, E16-E17, E19-E20, E22-E23

Data Acquisition Card Cage

Table A.2-4 Data Acquisition Card Cage Jumpers

Card	Jumpers
Pulse Generator and Clock R	X1 through X4
Pulse Pileup Det./TOF	E1-E2, E20-E21
Mux and Scope Interface	E1-E2, E3-E4
Quad PSH 1	E2-E4
Quad PSH 2	E2-E4
Peak ADC and PSH Control	E1-E2, E6-E7
Data Lister Out	E5-E6
Prism and Sort Window Test	None
Bitmap and Sort Decision	None
Interface and Scaler R	E1-E5, E2-E6, E3-E7
Sort Delay R3	None

Table A.2-4 Data Acquisition Card Cage Jumpers *(Continued)*

Card	Jumpers
Sort Oscillator R2	None
Sort Output R	None

Jumpers for Line Voltages

Table A.2-5 Line Voltage Jumpers

100 Vac input to main system transformer	TB9-2B to TB9-4B (jumper cable) TB9-7B to TB9-9B (jumper cable) TB9-1B to TB9-2B (jumper brass) TB9-6B to TB9-7B (jumper brass)
115 Vac input to main system transformer	TB9-2B to TB9-4B (jumper cable) TB9-3B to TB9-5B (jumper cable) TB9-1B to TB9-2B (jumper brass) TB9-5B to TB9-6B (jumper brass)
230 Vac input to main system transformer	TB9-1B to TB9-2B (jumper brass) TB9-3B to TB9-4B (jumper brass) TB9-5B to TB9-6B (jumper brass)
240 Vac input to main system transformer	TB9-1B to TB9-2B (jumper brass) TB9-4B to TB9-8B (jumper cable) TB9-6B to TB9-10B (jumper cable)
100 Vac input to laser auto transformer	TB10-1B to TB10-4B (jumper cable) TB10-2B to TB10-8B (jumper cable) TB10-6B to TB10-9B (jumper cable) TB10-8B to TB10-10B (jumper cable) TB10-4B to TB10-5B (jumper brass) TB10-6B to TB10-7B (jumper brass) Remove TB10-1B to TB10-10B (jumper cable)
115 Vac input to laser auto transformer	TB10-1B to TB10-10B (jumper cable)
230 Vac input to laser auto transformer	TB10-1B to TB10-4B (jumper cable) TB10-6B to TB10-9B (jumper cable) TB10-8B to TB10-10B (jumper cable) TB10-7B to TB10-8B (jumper brass) Remove TB10-1B to TB10-10B (jumper cable)
240 Vac input to laser auto transformer	TB10-1B to TB10-3B (jumper cable) TB10-6B to TB10-9B (jumper cable) TB10-8B to TB10-10B (jumper cable) TB10-7B to TB10-8B (Jumper brass) Remove TB10-1B to TB10-10B (jumper cable)

A.3 OPTICAL SETTINGS

Collection Optics Upgrade

Crystal drive: 0.0%
Deflection: 0.0%.

Sort Waveform Verification

Drop frequency: 32 kHZ, so each droplet period is 31.25 microseconds.
Crystal drive:100%.
Deflection amplitude: 100%.
Sort Test: ON
Sort Right Enable: ON
Sort Left Enable: ON.
Stream control sort pulse baseline: 0 V.
Front Porch length = Back Porch length - 31.25 microseconds.

Center level length = number of droplets to sort:1.
Front and Back porches: 0 to 100% of Center level.

Crystal Drive Test Procedure

Wave form: sinusoidal, measuring 160 V peak to peak ±5 V with little distortion.

Crystal Drive Adjustment Procedure

Crystal drive:100%
Drop frequency: 32 kHz.
Adjust R84 on the Sort Oscillator card for 8.5 V peak to peak.
Adjust R118 on the Sort Output card for 160 V ± 5 V peak to peak signal.

Sort Pulse Amplitude Test

Drop frequency: 32 kHz.
Sort Test: ON.
Sort Left Enable: ON.
Sort Right Enable: ON.
Sort Right counter: OFF.
Left Stop counters: OFF.
Deflection: 100%.
Drops sorted: 3.
Signal baseline: 0 Vdc.
Amplitude of center level from the baseline: 80 V ± 2.5 V.
Negative-going pulse: symmetrical with positive-going pulse.
Front and Back porches: varied from 0% to 100%.
Front and Back porches: back to reference values.

Front porch at 80%,
Rear porch at 20%.

Power Supplies

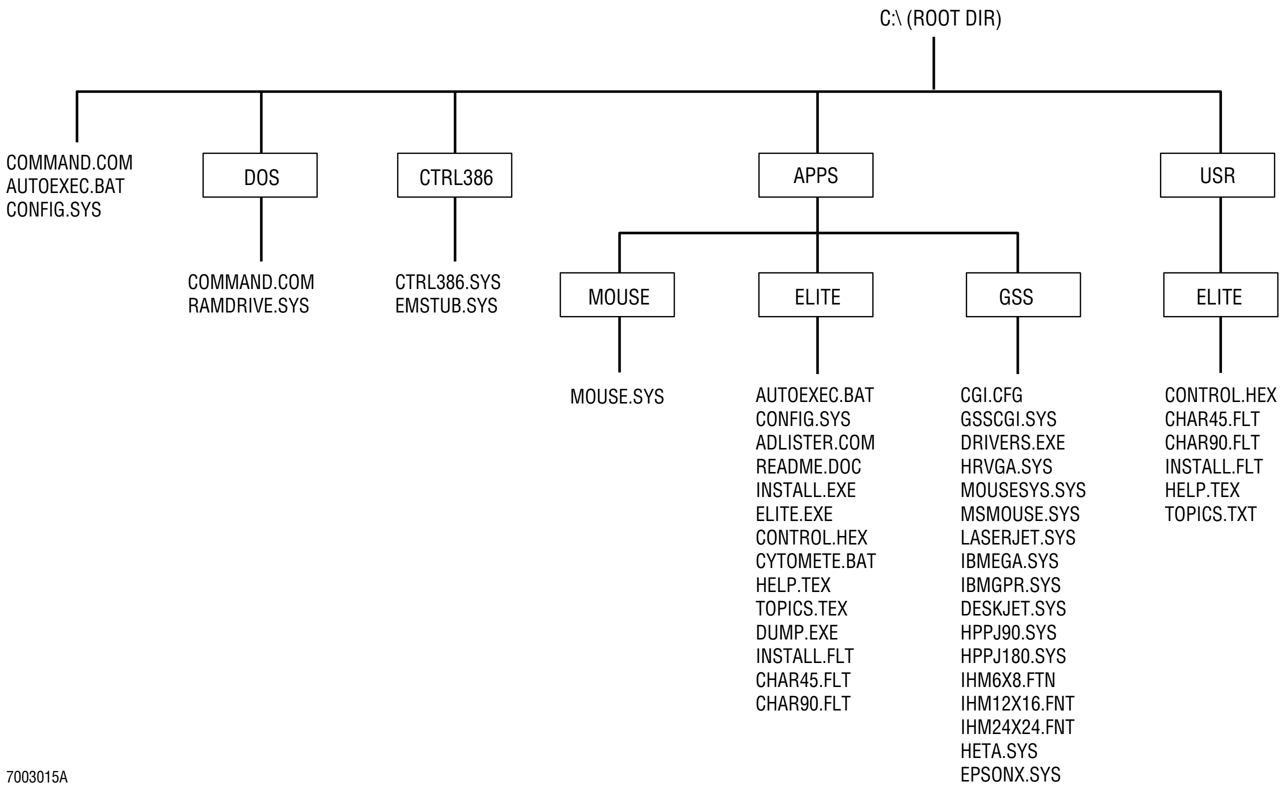
Table A.3-1 Power Supply Adjustments

Fiber Optics supply	Turn to minimum intensity.
Switcher supply	Adjust for 5.25 Vdc ± 0.01 Vdc between yellow and black wires.
15 V 0.25 A (PMT) supply	Adjust for +15.0 ±0.05 Vdc on orange wire; -15.0 ±0.05 Vdc on green wire. Both referenced to black wire.
15 V 5.0 A supply	Locate the Pulse Generator and Clock card in the Data Acquisition (lower) backplane far left slot, and adjust the larger ±15 Vdc Analog Power Supply using a DVM as follows: **Black DVM Lead** / **Red DVM Lead** / **DVM Result** Black test point / Orange test point / +15.0 V ±0.05 Vdc Black test point / Green test point / -15.0 V ±0.05 Vdc
15V 3.4 A supply	Adjust the ±15 Vdc 3 A power supply (connected to Bertans) with DVM connected to the HV backplane at P13 on top Bertrans backplane. Put your black DVM lead on the black/white wire. Probe the green wire with the red DVM lead, and adjust for -15.1 V ±0.05 Vdc. Move the red DVM lead to the orange wire. Adjust for +15.1 V ±0.05 Vdc.
90 V power supply	Adjust the power supply using the DVM as follows: **Black DVM Lead** / **Red DVM Lead** / **DVM Result** -OUT, Upper Supply / +OUT, Upper Supply / +90 Vdc ±2 Vdc -OUT, Lower Supply / +OUT, Lower Supply / +90 Vdc ±2 Vdc
Deflection power supply	Not adjustable. Test at test points: • J2- black = ground • J3 - blue = -2.3 to -3.0 Vdc • J4 - red = +2.3 to +3.0 Vdc

B.1 LOCATION OF SOFTWARE FILES

Figure B.1-1 shows the location of software files.

Figure B.1-1 File Paths



The following list is a composite of the abbreviations, acronyms, and reference designators used in this manual. When the same abbreviation (or reference designator) is used for more than one word (or type of component), all meanings relevant to this manual are included, separated by semicolons.

SYMBOL

λ = wavelength of laser

Δ - change in; difference between

Ω - ohm

> - greater than

< - less than

- - subtract; negative

+ - plus

± - plus or minus

= - equal to

µ - micron

µA - micro amperes

µf - microfarad

µL - microliter

µm - micrometer

µsec - microsecond

°C - degrees Celsius

°F - degrees Fahrenheit

A

A - ampere

A/D - analog to digital

ac - alternating current

adpt - adapter

adj - adjustment

amp - amplifier

Ar - Argon

assy - assembly

auto - automatic

aux - auxiliary

avg - average

B

BK - blocking

BMWDTH - beam width

BNC connector - bayonet Neil-Concelman; connector

BP - band pass

Btu - British thermal units

C

C - centigrade; capacitor

CAL - calibration

cbl - cable

cfm- cubic foot per minute

Ch - channel

CK - choke

CL - cylinder

cm - centimeter

Coin - coincidence

Coin. Abrt. - coincidence abort

CPU - central processing unit

CRT - cathode ray tube

CV - coefficient of variation

D

D - degree; diameter of laser beam

D/A - digital to analog

DAC - digital to analog converter

dc - direct current

DCN - document control number

det - detector

DI - dichroic

diam - diameter

DL - dichroic long pass

DMS - data management system

DS - dichroic short pass

DUAL FL - Dual FL SW-R card

DVM - digital voltmeter

E

ea - each

EPROM - erasable programmable read-only; memory

ESD - electrostatic discharge

F

f = focal length of lens

F - Fahrenheit; function; fuse

FALS - forward angle light scatter

FF - feed-through fitting

FIFO - first in, first out

FL - filter; fluorescence

FPW - full pulse width

FRU - field-replaceable unit

FS - forward scatter

ft - foot

FT - T-fitting

FU - fitting union

FY - Y-fitting

G

g - gram

GA - gauge

gal - gallon

GIG - gigabyte

GND - ground

H

hd - head

HeCd - helium cadmium

HeNe - helium neon

Hg - mercury

ht - height

HV- high voltage; high volume

Hz - Hertz

I

IA - aperture current

i.d. - inside diameter

ID - identification

in. - inches

I/O - input/output

IR - infrared

K

K - constant; thousand

KΩ - kilohm

KB - kilobytes

kg - kilograms

kHz - kilohertz

kV - kilovolt

kW - kilowatt

kPa -kilo Pascal

L

l - long

L - left; length; liter; lock

laser - light amplification by stimulated emission of radiation

lb - pound

LCD - liquid crystal diode

LED - light emitting diode

Li - lithium

lm - lumen

LP - long pass

LT - digiscope lower trace BNC connector

LV - low volume

LWR.BM - lower beam

M

m - meter

mA - milli-amperes

max - maximum

MB - megabyte

MCB - Motor Control (board) card

MEM - memory

MF - manifold

min - minute
misc - miscellaneous
MF - manifold
MHz - mega Hertz
min - minimum; minute
mL - milliliter
mm - millimeter
msec - microsecond
mV - millivolts
mW - milliwatt

N
N/A - not applicable
NB -
ND - neutral density
nm - nanometer

O
o.d. - outside diameter
op amp - operational amplifier
oz - ounces

P
param - parameter
PC - personal computer
PCB - printed circuit board (card)
pf -
PGEN - Pulse Generator and Clock R card
PK SCAT - Peak Scatter/Mux SW-R card
PM - pump
PMI - preventive maintenance
PMT - photomultiplier tube
PMT GA - PMT Gated Amp
PN - part number
PNEU - pneumatics
pot - potentiometer
ppm - parts per million
PPU - pulse pileup
preamp - preamplifier
PROM - programmable read-only memory
PS - power supply
PSH - peak sense and hold
psi - pounds per square inch
PVC - polyvinylchloride
PWR - power

Q
QPSH&H - quad peak sense and hold
QUAD 7 DLY - Quad 7 Microsecond Delay card
QUAD 20 DLY - Quad 20/40/60 Microsecond Delay card

R
R - potentiometer; resistor; right; research
RAM - random access memory
REF - reference
REG - regulator
RF - radio frequency
RG - pressure regulator
ROM - read only memory

S
Scat - scatter
Scat/Aux - Scat/Aux Gated Amp
SCSI - small computer system interface
SD - standard deviation
Sensor I/F - Sensor Interface
SIMM - single in-line memory module
SL - solenoid
SN - serial number
SOL - solenoid
SP - short pass; stretched pulse
SS - side scatter
SUP - supply
SVGA - super video graphics adapoer
SVP - system verification procedure
SW - switch
SW-R -

sync - synchronized

T

TEMP - temperature

TOF - time of flight

TP - test point

trans - translator

TRIG - trigger

U

UL - Underwriters Laboratory

UNC - unified coarse thread

UNS - unified special thread

UPP.BM - upper beam

UT - digiscope upper trace BNC connector

UV - ultraviolet

V

V - volt

Vac - voltage alternating current

vac - vacuum

VC - vacuum chamber

Vdc - voltage direct current

VGA - video graphics adaptor

VL - valve

volt - voltage

vs. - versus

W

W - watt

w/ - with

WM - wire marker

w/o - without

wt - weight

X

x - multiply by

xmit - transmit

Numerics

A

B

C

D

M

N

O

P

Q

R

T

U

V

W

Z

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